PREPARATION AND STRUCTURE-ACTIVITY RELATIONSHIPS OF SOME 6α-SUBSTITUTED PENICILLINS

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The influence on the antibacterial activity of introducing a 6α-methoxy group into carbenicillin, and various 6α-substituents into sulbenicillin and piperacillin was examined. Further variations of the side chain aryl group were examined in the 6α-methoxy substituted series. This led to the identification of disodium 6β-(D,L-2-carboxy-2-thien-3-ylacetamido)-6α-methoxypenicillanate (**5b**) as a β-lactamase stable derivative with useful activity against *Enterobacteriaceae*, and disodium 6β-[D-2-(4-aminophenyl)-2-sulfoacetamido]-6α-methoxypenicillanate (**6e**) with slightly lower activity against the *Enterobacteriaceae* but more active against *Pseudomonas aeruginosa*.

In the early 1970's workers at the Lilly Research Laboratories[1] and at Merck Sharp and Dohme[2] independently discovered the cephamycins. This group of compounds is related to the cephalosporins but contains a 7α-methoxy substituent that improves stability to bacterial β-lactamases although often at the expense of intrinsic antibacterial activity. Since that time there have been many reports of 7α-substituted cephalosporins but only a few compounds have achieved the balance between antibacterial potency and β-lactamase stability required for clinical utility[3].

Much less attention has been paid to the 6α-substituted penicillins, possibly because early reports were not encouraging[4]. For example, introduction of a 6α-methoxy group into benzylpenicillin[5], phenoxymethylpenicillin[5], ampicillin[6], mecillinam[7] and azlocillin[8] resulted in compounds with little or no antibacterial activity. Indeed CAMA and CHRISTENSEN[9], discussing 6-substituents, stated that 'even the 6α-methoxy group on penicillins does not lead to useful compounds'.

This report discusses the preparation of a number of novel 6α-substituted penicillins and their structure-activity relationships.

The 6α-substituted penicillins described in this paper were prepared by the two general methods outlined in Scheme 1. In the first, benzyl 6β-amino-6α-methoxypenicillanate (**1**), prepared as required[10] from benzyl 6β-amino-6α-methylthiopenicillanate (**7**)[6,10], was acylated with an acid chloride (**2**) in the presence of an organic base. Purification followed by removal of the benzyl ester and any protecting groups in the side chain gave the 6α-methoxypenicillins **5** and **6**. Towards the conclusion of this work an improved method for the transformation of **7** to **1** was described[11]. Crude **1**, prepared by this method, could be readily crystallized and was then stable to storage at <5°C.

The other mehod, B, involved acylation of **7** with either an acid chloride (**2**) or the acid (**8**) in the

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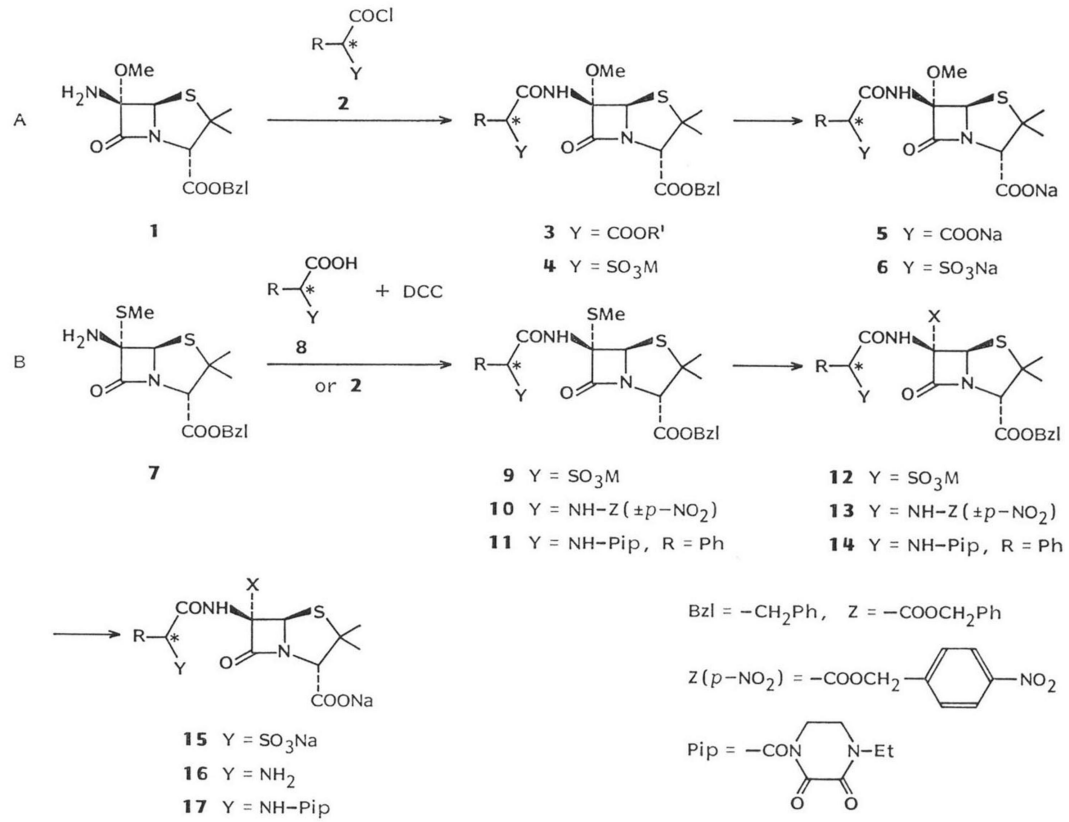
Scheme 1.



presence of dicyclohexylcarbodiimide (DCC). The intermediate 6α-methylthiopenicillins **9**~**11** were then deprotected, or the 6α-substituent displaced by a nucleophile in the presence of mercuric acetate and then deprotected, to give the penicillins **15**~**17** with the 6α-substituents described below.

All the compounds prepared can exist as a pair of stereoisomers, epimeric in the 6β-acyl side chain at 2'(*). The aim throughout this work was to obtain the stereoisomer derived from the side chain with D configuration wherever possible. This was achieved either by using resolved side chains or by separating the isomers at an intermediate stage, **4**, **9** or **13**. The assignment of the configuration in the 6β-acyl side chains was based on NMR data[12] and confirmed in one case (**4e**, M=$HNEt_3$) by X-ray analysis (Fig. 1).

In the preparation of carboxy 6α-methoxypenicillins (**5**) by method A, the second of the carboxyl groups of the side chain acids was protected. Trimethylsilyl, **a** and **b**, benzyl, **d**, **e** and **f**, and phenyl, **c**, esters were used. Additionally the hydroxyl group, **d**, was protected as its benzyl ether and the amino group, **e**, as its benzyloxycarbonyl derivative. The benzyl derived protecting groups were removed by hydrogenation followed where necessary by hydrolysis of the side chain ester with aqueous base to give **5a**~**f**.

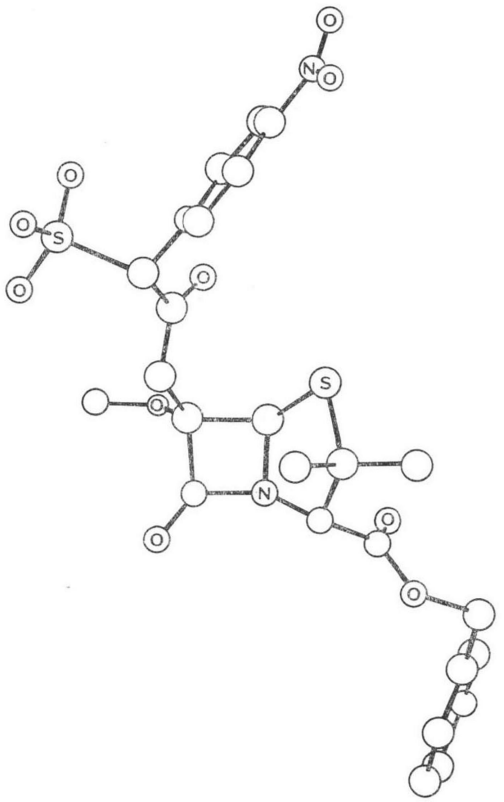
The preparation of substituted sulbenicillin analogues, **6** and **15**, required a versatile method of preparing 2-sulfo substituted acetyl chlorides **2** (Y=SO_3H). Sulfonation of substituted acetyl chlorides with sulphur trioxide-dioxane complex, Scheme 2, proved to be such a method provided that the R group was not too electron-rich, (*e.g.* methoxyphenyl or thienyl).

Acid chlorides prepared in this way were used, without isolation, to acylate **1** or **7** in the presence of triethylamine or *N*-methylmorpholine. The intermediate esters **4** and **9** were obtained as the sulfonic acid triethylammonium or *N*-methylmorpholinium salts and as a mixture of epimers. These salts were purified by chromatography on silica gel and this, in several cases, gave separation of the stereoisomers. Often the more polar isomer could be crystallized and was found to be derived from the side-chain with D configuration.

The limitations of the sulfonation reaction dictated different protecting groups for the hydroxyl, **6d**, and amino groups, **6e**, from those used in the carboxy series. The hydroxyl group was masked as its acetate ester and hydrolysed with citrus acetylesterase at the final step. The amino group was obtained by hydrogenation of the nitro substituted intermediate, **4e**.

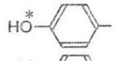
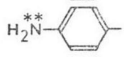
The penicillins, **6a**~**f** and **15a**~**g**, were obtained as their sodium salts by ion exchange before or after hydrogenation of the penicillin esters. Removal of the benzyl ester to give **6g** could not be performed by hydrogenation as this also removed bromine. However, the stability conferred on the β-lactam ring by the 6α-methoxy group allowed the removal of the benzyl ester by alkaline hydrolysis.

Fig. 1. A view of the X-ray crystal structure of **4e**. (Hydrogen atoms and the cation, $H\overset{+}{N}Et_3$, are not shown.)



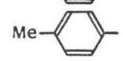
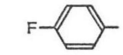
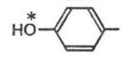
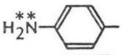
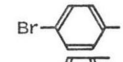
2- and 3-thienylacetyl chlorides could not be regiospecifically mono-sulfonated so an alternative

Table 1. 6α-Methoxycarboxypenicillins **5**.

	R	2′-Configuration	Carboxyl protection R′
a		D,L	$Si(Me)_3$***
b		D,L	$Si(Me)_3$***
c		D,L	Ph
d		D,L	Bzl
e		D,L	Bzl
f		D,L	Bzl

* Protected as the benzyl ether.
** Protected as the benzyloxycarbonyl derivative.
*** Hydrolysed to R′=H during work-up.

Table 2. 6α-Substituted sulbenicillin analogues, **6** and **15**.

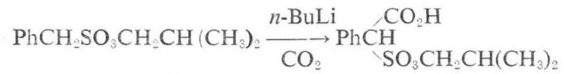
	6α-Methoxy derivatives **6**		6α-Substituted derivatives **15** (R=Ph)		
	R	2′-Configuration		X	2′-Configuration
a		D	**a**	–SMe	D
b		D,L	**b**	–OH	D
c		D,L	**c**	–OEt	D
d		D,L	**d**	–NHMe	D
e		D	**e**	$-NMe_2$	D
f		D,L	**f**		D
g		D,L	**g**	–NHOMe	D
h		D,L			
i		D,L			

* Protected as the acetate.

** For **4e** R=  .

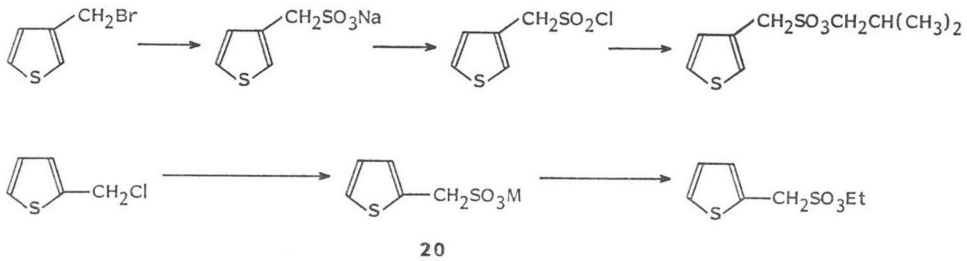
Scheme 2.

Scheme 3.



18 **19**

Scheme 4.



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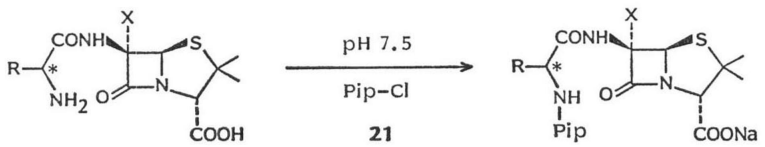
was sought. 2-Methylpropyl phenylmethanesulonate (**18**) could be readily converted to a suitable side chain acid **19** (Scheme 3). The novel synthesis of the corresponding thienylmethanesulfonates was performed as detailed in Scheme 4. In contrast to its 3-thienyl isomer, sodium 2-thienylmethanesulfonate (**20**, M=Na) could not be converted to its acid chloride, nor could its silver salt (M=Ag) be alkylated by 2-methylpropyl bromide. The ethyl sulfonate was prepared by alkylation of the cetyltrimethylammonium salt (**20**, $M=Me_3NC_{16}H_{33}$), with triethyloxonium tetrafluoroborate. The sulfonates were then converted to their carboxylic acid derivatives and elaborated to the penicillins using method A. Aqueous hydrolysis of the sulfonates at the final stage gave **6h** and **i**.

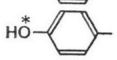
Two variations of method B were used to prepare 6α-substituted piperacillin analogues **17**. When

Scheme 5.



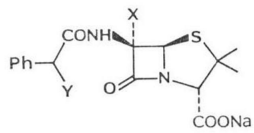
16 17

Table 3. 6α-Substituted piperacillin analogues **17**.

	6α-Methoxy derivatives **17** (X=OMe)			6α-Substituted derivatives **17** (R=Ph)	
	R	2′-Configuration		X	2′-Configuration
a		D	f	-OH	D
b		D	g	-OEt	D
c		D	h	-SMe	D
d		D	i	-NHMe	D
e		D, L	j	$-NMe_2$	D
			k	$-N\langle^{OH}_{Me}$	D
			l	-NHOMe	D

* Protected as the benzyloxycarbonyl derivative.

Table 4. *In vitro* antibacterial activity (MIC, μg/ml)[c].



	22	**5a**	**23**	**15b**	**6a**	**24**	**17f**	**17a**
X	H	OCH_3	H	OH	OCH_3	H	OH	OCH_3
Y	CO_2Na	CO_2Na	SO_3Na	SO_3Na	SO_3Na	NH-pip	NH-pip	NH-pip
Escherichia coli NCTC 10418	5	10	10	2.5	5	0.5	5	10
E. coli JT4[a]	>100	10	>100	>100	10	>100	>100	50
E. coli JT425[b]	10	10	25	50	5	10	>100	50
Pseudomonas aeruginosa NCTC 10662	25	>100	25	>100	>100	5	>100	>100
P. aeruginosa Dalgleish[a]	>100	>100	>100	>100	>100	>100	>100	>100
Klebsiella pneumoniae A	10	2.5	25	10	1.0	2.5	2.5	25
Serratia marcescens US32	25	25	25	10	10	1.0	25	25
Enterobacter cloacae N1	2.5	2.5	2.5	2.5	2.5	2.5	10	25
Proteus mirabilis C977	1.0	5	2.5	2.5	2.5	0.1	1.0	10
P. mirabilis 889[b]	>100	5	>100	10	5	100	>100	10
P. rettgeri	5	5	2.5	2.5	2.5	1.0	25	50
Staphylococcus aureus Oxford	0.5	>100	2.5	50	>100	0.5	1.0	>100
Streptococcus pyogenes CN10	0.2	>100	0.1	2.5	50	0.05	0.5	10

[a] β-Lactamase producing strain (plasmid mediated).

[b] β-Lactamase producing strain (non-plasmid mediated).

[c] Determined by serial dilution in nutrient agar containing 5 % v/v defibrinated horse blood, inoculum 0.001 ml of an overnight broth culture (approximately 10^6 cfu).

Table 5. *In vitro* activity of 6α-methoxycarboxypenicillins **5** (MIC, μg/ml)[c].

	a	b	c	d	e	f
E. coli NCTC 10418	10	2.5	50	25	10	10
E. coli JT4[a]	10	5	100	25	25	25
E. coli JT425[b]	10	2.5	25	25	25	10
P. aeruginosa NCTC 10662	>100	>100	>100	50	>100	>100
P. aeruginosa Dalgleish[a]	>100	>100	>100	50	>100	>100
K. pneumoniae A	2.5	1.0	5	5	5	5
S. marcescens US32	25	10	>100	>100	50	10
E. cloacae N1	2.5	5	50	10	10	10
P. mirabilis C977	5	5	10	10	10	5
P. mirabilis 889[b]	5	2.5	10	10	10	10
P. rettgeri	5	1.0	5	10	25	5
S. aureus Oxford	>100	>100	>100	>100	>100	>100
S. pyogenes CN10	>100	25	>100	>100	25	25

[a, b, c]: See footnotes in Table 4.

a benzyloxycarbonyl- or 4-nitrobenzyloxycarbonyl protected substituted glycine was used, deprotection gave the amino penicillins **16a**~**g**. Acylation of these with 2,3-dioxo-4-ethylpiperazin-1-ylcarbonyl chloride (**21**) readily gave the 6α-substituted piperacillin analogues **17a**~**g** (Scheme 5). The penicillins in which R is phenyl, and 4-hydroxyphenyl, with the hydroxyl protected by a benzyloxycarbonyl group, were prepared form the resolved glycines.

The other 6α-substituted piperacillins **17h**~**l**, were prepared from the intact side chain. D-Phenylglycine was acylated with 2,3-dioxo-4-ethylpiperazin-1-ylcarbonyl chloride (**21**) and the acid produced, (**8**, R=Ph, Y=NH-pip) used to acylate **7**. This, however, gave some racemization but the intermediate **11** could be obtained as the single stereoisomer following chromatography and crystallization. This was then used to prepare the penicillins **17h**~**l**.

Results and Discussion

The introduction of a 6α-methoxy group into carbenicillin (**22**), sulbenicillin (**23**) and piperacillin (**24**) gave three derivatives (Table 4: **5a**, **6a**, **17a**) which showed considerable improvement in activity against β-lactamase-producing strains of *Escherichia coli* and *Proteus mirabilis* compared to their 6α-unsubstituted analogues (**22**, **23**, **24**). Against other Gram-negative bacteria, however, the activities of the 6α-methoxy penicillins were similar or less than that of carbenicillin, sulbenicillin or piperacillin. In particular their activities were much reduced against *Pseudomonas aeruginosa* and against Gram-positive bacteria.

The 6α-hydroxypenicillins (Table 4: **15b**, **17f**) displayed a level of activity intermediate between that of the 6α-methoxypenicillins (**6a**, **17a**) and the unsubstituted compounds (**23**, **24**). All the other 6α-substituted derivatives described (**15a**, **15c**~**g**, **17g**~**l**) showed weak or no activity (MIC values >25 μg/ml) against the bacteria in Table 4.

Modification of the 6β-acyl group in 6α-methoxycarboxypenicillins (**5**) produced a number of compounds (Table 5) which were stable to β-lactamases but varied in their degree of potency. The 3-thienyl derivative (**5b**) was the most active compound against the *Enterobacteriaceae* and this was developed subsequently as temocillin[18]. The 4-hydroxyl compound (**5d**) was also of some interest in that it showed some activity against *P. aeruginosa* although the activity against the *Enterobacteriaceae* was less than that of temocillin.

Table 6 shows a number of 6α-methoxysulbenicillin analogues (**6**). The compounds displayed a similar spectrum of activity to temocillin but in this series the 3-thienyl analogue (**6h**) showed no advantage over the phenyl compound (**6a**). The 4-hydroxy (**6d**) and in particular the 4-amino (**6e**)

Table 6. *In vitro* activity of 6α-methoxysulbenicillin derivatives **6** (MIC, μg/ml)[c].

	a	b	c	d	e	f	g	h	i
E. coli NCTC 10418	5	>100	25	25	5	10	10	10	10
E. coli JT4[a]	10	>100	50	25	10	25	50	10	25
E. coli JT425[b]	5	>100	50	25	5	10	25	10	10
P. aeruginosa NCTC 10662	>100	>100	>100	50	25	100	>100	>100	>100
P. aeruginosa Dalgleish[a]	100	>100	>100	50	25	50	>100	>100	>100
K. pneumoniae A	1.0	25	5	5	2.5	10	5	5	10
S. marcescens US32	10	>100	50	50	25	25	>100	25	50
E. cloacae N1	2.5	25	10	10	5	25	5	10	10
P. mirabilis C977	2.5	25	25	25	5	25	5	10	10
P. mirabilis 889[b]	5.0	25	10	10	5	25	5	10	25
P. rettgeri	2.5	25	10	25	5	25	5	10	10
P. aureus Oxford	>100	50	>100	>100	>100	>100	>100	100	>100
S. pyogenes CN10	50	25	50	25	25	>100	50	25	100

[a], [b], [c]: See footnotes in Table 4.

Table 7. *In vitro* activity of 6α-methoxypiperacillin derivatives **17** (MIC, μg/ml)[c].

	a	b	c	d	e
E. coli NCTC 10418	10	5	5	25	25
E. coli JT4[a]	50	25	10	50	10
E. coli JT425[b]	50	100	5	100	100
P. aeruginosa NCTC 10662	>100	>100	>100	>100	>100
P. aeruginosa Dalgleish[a]	>100	>100	>100	>100	>100
K. pneumoniae A	25	1.0	10	2.5	0.5
S. marcescens US32	25	10	10	50	50
E. cloacae N1	25	100	50	50	50
P. mirabilis C977	10	5	2.5	10	10
P. mirabilis 889[b]	10	1.0	1.0	5	5
P. rettgeri	50	100	50	100	50
S. aureus Oxford	>100	>100	10	>100	>100
S. pyogenes CN10	10	5	1.0	2.5	50

[a], [b], [c]: See footnotes in Table 4.

derivatives in addition showed activity (MIC 25 μg/ml) against *P. aeruginosa* including β-lactamase-producing strains such as Dalgleish, that are highly resistant to sulbenicillin. Thus in 6α-methoxy-4-aminosulbenicillin (**6e**) we have a derivative which has slightly reduced activity compared to temocillin against *Enterobacteriacea*, exhibits improved anti-*Pseudomonas* activity but in common with temocillin lacks activity against Gram-positive organisms.

6α-Methoxypenicillins with the acylated amino substituent (**17**) were of less biological interest (Table 7). 6α-Methoxypiperacillin (**17a**) was only moderately active against Gram-negative organisms; moreover, variation of the aryl group in the 6β-acyl side chain (Table 7: **17b**~**e**) failed to give significantly improved activity. In addition the series was not active against *P. aeruginosa* or, with the exception of **17c**, against Gram-positive organisms.

Our results demonstrate that in contrast to the conclusion of CAMA and CHRISTENSEN[6] it is possible to obtain 6α-substituted penicillins with significant antibacterial activity in addition to possessing stability to β-lactamases.

Experimental

IR spectra were recorded on a Perkin Elmer 197, 459 or 983 machine for 0.4 % w/w sample in a 300 mg KBr disc unless otherwise stated. ^{1}H NMR spectra were recorded at 60 MHz on a Varian EM

360, at 90 MHz on a Perkin Elmer R32 and at 250 MHz on a Brucker WM250 instrument, for solutions in [$(CD_3)_2CO$], with tetramethylsilane as internal standard, unless otherwise stated.

Solutions were dried over anhydrous magnesium sulfate and solvents were removed by evaporation under reduced pressure using a rotary evaporator.

Hydrogenation of benzyl protecting groups was performed in the presence of an equal weight of 10% palladium on carbon at atmospheric temperature and pressure, followed by HPLC and the catalyst removed by filtration through Celite.

Compounds used for antibacterial testing were all essentially single materials, or pairs of stereoisomers, analyzed by reverse phase HPLC, Waters Associates Inc. μBondapak C-18 column eluted with methanol in 0.05 M ammonium acetate (pH 4.5) or with acetonitrile in 0.05 M sodium acetate (pH 5.0) and detected by UV absorption at 240 nm. Where possible the identity of the final products was confirmed by positive ion fast atom bombardment mass spectrometry[14] using a VG Analytical ZAB reverse geometry mass spectrometer fitted with an Ion Tech fast atom bombardment gun. Samples were prepared by dissolving the penicillin in water and/or methanol and mixing with glycerol on the target.

Purification of Benzyl 6β-Amino-6α-methoxypenicillanate (**1**)

Crude **1** (5 g), prepared from **7** with cupric acetate[11], was crystallized from isopropyl acetate - cyclohexane (2.0 g). MP 78~79°C, ref 10. MP 40~43°C. ^{1}H NMR (60 MHz, $CDCl_3$) δ 1.40 and 1.53 (6H, 2s, 2×$2CH_3$), 2.30 (2H, br s, NH_2), 3.43 (3H, s, OCH_3), 4.43 (1H, s, 3-H), 5.17 (2H, s, OCH_2), 5.32 (1H, s, 5-H), 7.33 (5H, s, Ph).

Anal Calcd for $C_{16}H_{20}N_2O_4S$: C 57.13, H 5.99, N 8.33, S 9.53.
Found: C 57.13, H 5.98, N 8.43, S 9.36.

Disodium 6β-(D,L-2-Carboxy-2-phenylacetamido)-6α-methoxypenicillanate (**5a**)

Phenylmalonic acid (3.6 g), thionylchloride (1.6 ml) and DMF (10 μl) in diisopropyl ether (50 ml) were heated at 45~50°C for 1 hour then evaporated to half volume. Diisopropyl ether (25 ml) and trimethylchlorosilane (2.6 ml) were added followed by hexamethyldisilazane (1.7 ml) in diisopropyl ether (5 ml) dropwise at 0°C. This acid chloride solution was added dropwise to **1** (6.72 g) and pyridine (4 ml) in THF (50 ml) at 0°C, stirred for 1 hour without cooling. The mixture was washed with 1 M HCl (50 ml) and extracted with 1 M $NaHCO_3$ (3×25 ml). The extracts were washed with ether (3×25 ml), acidified to pH 3 and extracted with dichloromethane (3×25 ml). The organic solution was washed with water (4×25 ml) to remove phenylmalonic acid, then dried and evaporated to give benzyl 6β-(D,L-2-carboxy-2-phenylacetamido)-6α-methoxypenicillanate (**3a**) as a foam, 5.9 g (50 %). ^{1}H NMR (60 MHz) δ 1.10 and 1.23 (6H, 2s, 2×$2CH_3$), 3.28 and 3.38 (3H, 2s, OCH_3, epimers), 4.33 (1H, s, 3-H), 4.58 (1H, s, 2'-H), 5.17 (2H, s, OCH_2), 5.57 (1H, s, 5-H), 7.33 (10H, s, 2×Ph), 8.17 and 8.27 (1H, 2s, 6β-NH, epimers), 9.70 (1H, br s, CO_2H).

3a (5.0 g) in ether (80 ml) was extracted with 0.5 M $NaHCO_3$ (3×20 ml) and the aqueous solution hydrogenated for 3 hours. The filtered solution was washed with ether (2×25 ml), acidified to pH 2.5 and extracted with ethyl acetate (3×25 ml). The extracts were washed with water (2×25 ml) and brine (20 ml), dried and evaporated to a foam (3.05 g) which, in acetone (10 ml), was treated with 1.85 M sodium 2-ethylhexanoate in 4-methylpentan-2-one (7.4 ml). The precipitate of **5a** was collected, washed with acetone and ether and dried, 2.88 g (64 %). IR ν_{max} cm^{-1} 3432, 1765, 1664, 1608, 1494, 1102. ^{1}H NMR (250 MHz, D_2O) δ 1.32, 1.41, 1.43 and 1.56 (6H, 4s, 2×$2CH_3$, epimers), 3.42 and 3.55 (3H, 2s, OCH_3, epimers), 4.26 and 4.28 (1H, 2s, 3-H, epimers), 4.61 and 4.67 (1H, 2s, 2'-H, epimers), 5.48 and 5.50 (1H, 2s, 5-H, epimers), 7.3~7.5 (5H, m, Ph).

Disodium 6β-(D,L-2-Carboxy-2-thien-3-ylacetamido)-6α-methoxypenicillanate (**5b**)

Compounds **3b** and **5b** were obtained from thien-3-ylmalonic acid using the procedure described for the preparation of **3a** and **5a**.

3b: Yield 69%. ^{1}H NMR (60 MHz) δ 1.23 and 1.28 (6H, 2s, 2×$2CH_3$), 3.33 and 3.38 (3H, 2s, OCH_3, epimers), 4.33 (1H, s, 3-H), 4.77 (1H, s, 2'-H), 5.20 (2H, s, OCH_2), 5.57 (1H, s, 5-H), 6.9~7.5 (8H, m, Ph and thienyl protons), 8.03 and 8.17 (1H, 2s, 6β-NH, epimers), 10.70 (1H, br s, CO_2H).

5b: Yield 27%. IR ν_{max} cm^{-1} 3426, 1764, 1672, 1605, 1498, 1097. ^{1}H NMR (250 MHz, D_2O)

δ 1.39, 1.42, 1.44 and 1.47 (6H, 4s, $2\times2CH_3$, epimers), 3.47 and 3.56 (3H, 2s, OCH_3, epimers), 4.28 and 4.29 (1H, 2s, 3-H, epimers), 4.67 and 4.73 (1H, 2s, 2′-H, epimers), 5.52 and 5.53 (1H, 2s, 5-H, epimers), 7.1~7.5 (3H, m, thienyl protons). FAB-MS m/z 459 (M+H, $C_{16}H_{17}N_2O_7S_2Na_2$).

Disodium 6β-[D,L-2-Carboxy-2-(4-methoxyphenyl)acetamido]-6α-methoxypenicillanate (**5c**)

Phenyl hydrogen 4-methoxyphenylmalonate (2.15 g) was heated under reflux in thionyl chloride (10 ml) for 2 hours and evaporated to dryness. This acid chloride in dichloromethane (50 ml) was added dropwise to **1** (2.0 g) and pyridine (1 ml) in dichloromethane (100 ml) at 0°C. After 2 hours the mixture was washed with 0.5 M HCl, water, 1 M $NaHCO_3$ and water then **3c** isolated by chromatography on silica gel eluting with ethyl acetate - light petroleum (bp 60~80°C), 1.47 g (41%). ^{1}H NMR (60 MHz, $CDCl_3$) δ 1.33 (6H, s, $2\times2CH_3$), 3.43 and 3.46 (3H, 2s, 6α-OCH_3, epimers), 3.80 (3H, s, OCH_3), 4.67 (1H, s, 2′-H), 5.22 (2H, s, OCH_2), 5.65 (1H, s, 5-H), 6.8~7.7 (14H, m, aromatic), 7.76 and 7.87 (1H, 2s, 6β-NH, epimers).

3c was hydrogenated in 5% aqueous ethanol using the method described for compound **5a** to give sodium 6β-[D,L-2-(4-methoxyphenyl)-2-phenoxycarbonylacetamido]-6α-methoxypenicillanate, 87% yield. ^{1}H NMR (60 MHz, free acid, $CDCl_3$) δ 1.22 and 1.32 (6H, 2s, $2\times2CH_3$), 3.42 and 3.47 (3H, 2s, 6α-OCH_3, epimers), 3.80 (3H, s, OCH_3), 4.43 (1H, s, 3-H), 4.95 (1H, s, 2′-H), 5.67 (1H, s, 5-H), 6.8~7.7 (9H, m, aromatic), 8.12 and 8.22 (1H, 2s, 6β-NH, epimers).

Sodium 6β-[D,L-2-(4-methoxyphenyl)-2-phenoxycarbonylacetamido]-6α-methoxypenicillanate (0.59 g) and sodium tetraborate decahydrate (0.52 g) in water (50 ml) were stirred for 2 hours and **5c** isolated using the procedure described for **5a**, 0.20 g (39%). ^{1}H NMR (60 MHz, D_2O) δ 1.12, 1.17, 1.20 and 1.23 (6H, 4s, $2\times2CH_3$, epimers), 3.27 and 3.37 (3H, 2s, 6α-OCH_3, epimers), 3.60 (3H, s, OCH_3), 4.08 (1H, s, 3-H), 4.30 and 4.38 (1H, 2s, 2′-H), 5.35 (1H, s, 5-H), 6.82 and 7.21 (4H, ABq, J=9 Hz, aromatic).

Disodium 6β-[D,L-2-Carboxy-2-(4-hydroxyphenyl)acetamido]-6α-methoxypenicillanate (**5d**)

3d was prepared from benzyl hydrogen 4-benzyloxyphenylmalonate using the procedure described for **3c**, 35% yield. ^{1}H NMR (60 MHz, $CDCl_3$) δ 1.25 (6H, s, $2\times2CH_3$), 3.33 and 3.43 (3H, 2s, OCH_3, epimers), 4.52 (1H, s, 3-H), 4.78 (1H, s, 2′-H), 5.05 (2H, s, OCH_2), 5.22 (4H, s, $2\times OCH_2$), 5.70 (1H, s, 5-H), 6.9~7.8 (19H, m, aromatic), 8.18 and 8.28 (1H, 2s, 6β-NH, epimers).

Prolonged hydrogenation of **3d** using the procedure described for **3c** gave **5d** in 13% yield. IR ν_{max} cm^{-1} 3410, 1770, 1675, 1605, 1100. ^{1}H NMR (90 MHz, DMSO-d_6) δ 1.36 (6H, s, $2\times2CH_3$), 3.23 and 3.29 (3H, 2s, OCH_3, epimers), 3.99 and 4.02 (1H, 2s, 3-H, epimers), 4.09 (1H, s, 2′-H), 5.29 and 5.31 (1H, 2s, 5-H, epimers), 6.3 (1H, br s, OH), 6.76 and 7.05 (4H, ABq, J=9 Hz, aromatic), 10.75 (1H, s, 6β-NH).

Disodium 6β-[D,L-2-(4-Aminophenyl)-2-carboxyacetamido]-6α-methoxypenicillanate (**5e**)

Benzyl hydrogen (4-benzyloxycarbonylaminophenyl)malonate (0.84 g) in dichloromethane was stirred with oxalyl chloride (0.5 ml) and DMF (4 μl) for 1 hour and evaporated to give the acid chloride. This was used to prepare **3e**, 53% yield, as described for **3c**. ^{1}H NMR (60 MHz, $CDCl_3$) δ 1.26 and 1.34 (6H, 2s, $2\times2CH_3$), 3.35 and 3.45 (3H, 2s, OCH_3, epimers), 4.40 (1H, s, 3-H), 4.65 (1H, s, 2′-H), 5.20 (6H, s, $3\times OCH_2$), 5.60 (1H, s, 5-H), 7.1~7.7 (19H, m, aromatic), 7.95 (1H, br s, CONH), 8.05 (1H, br s, CONH).

Hydrogenation as described for **3d** provided **5e**, 68% yield. IR ν_{max} cm^{-1} 1765, 1670, 1600. ^{1}H NMR (90 MHz, D_2O - $(CD_3)_2CO$) δ 1.50 (6H, br s, $2\times2CH_3$), 3.60 (3H, s, OCH_3), 4.30 (1H, s, 3-H), 5.70 (1H, s, 5-H), 6.6~7.5 (4H, m, aromatic).

Disodium 6β-(D,L-2-Carboxy-2-thien-2-ylacetamido)-6α-methoxypenicillanate (**5f**)

3f was prepared from benzyl hydrogen thien-2-ylmalonate in 52% yield by the procedure described for **3c**. ^{1}H NMR (60 MHz, $CDCl_3$) δ 1.22 (6H, s, $2\times2CH_3$), 3.25 (3H, s, OCH_3), 4.32 (1H, s, 3-H), 4.92 (1H, s, 2′-H), 5.15 and 5.20 (4H, 2s, $2\times OCH_2$), 5.58 (1H, s, 5-H), 6.9~7.5 (3H, m, thienyl protons), 7.30 (10H, s, 2×Ph), 7.75 and 7.85 (1H, 2s, 6β-NH, epimers).

Hydrogenation as described for **3d** gave a mixture of **5f** and sodium 6β-(D,L-2-benzyloxycarbonyl-2-thien-2-ylacetamido)-6α-methoxypenicillanate. This mixture in water was maintained at pH 9.5

with 2.5 M NaOH for 3 hours, adjusted to pH 6.5 with Amberlite IR-120 (H^+) resin, filtered, washed with ether and freeze-dried to give **5f**, 60% yield. IR ν_{max} cm^{-1} 1765, 1670, 1605, 1095. ^1H NMR (60 MHz, D_2O) δ 1.41 (6H, br s, $2\times2CH_3$), 3.40 and 3.50 (3H, 2s, OCH_3, epimers), 4.20 (1H, s, 3-H), 4.89 (1H, s, 2'-H), 5.60 (1H, s, 5-H), 6.8~7.5 (3H, m, thienyl protons).

Disodium 6α-Methoxy-6β-(D-2-phenyl-2-sulfoacetamido)penicillanate (**6a**)

1 M Sulphur trioxide dioxane complex in 1,2-dichloroethane (30 ml) was added dropwise to phenylacetyl chloride (3.1 g) in 1,2-dichloromethane (50 ml) and the mixture stirred overnight. This solution of 2-phenyl-2-sulfoacetyl chloride was added dropwise to **1** (6.72 g) and *N*-methylmorpholine (10 ml) in dichloromethane (40 ml) at 0°C. After 1 hour the reaction mixture was washed with water (3×100 ml), concentrated and chromatographed on silica gel eluting with methanol in chloroform containing 0.1 % *N*-methylmorpholine to give, first benzyl 6α-methoxy-6β-(L-2-phenyl-2-sulfoacetamido)-penicillanate *N*-methylmorpholinium salt, 1.76 g (14 %). ^1H NMR (60 MHz) δ 1.36 and 1.55 (6H, 2s, $2\times2CH_3$), 2.30, 3.0~3.3 and 3.6~3.9 (11H, s and 2m, *N*-methylmorpholine), 3.55 (3H, s, OCH_3), 4.60 (1H, s, 3-H), 5.00 (1H, s, 2'-H), 5.30 (2H, s, OCH_2), 5.60 (1H, s, 5-H), 7.3~7.9 (10H, m, $2\times$Ph), 9.45 (1H, s, 6β-NH). **4a** (M=*N*-methylmorpholinium) was then eluted, 3.76 g (30 %) which crystallized from acetone, mp 166~167°C. IR ν_{max} cm^{-1} 3238, 3195, 1778, 1742, 1684. ^1H NMR (90 MHz, DMSO-d_6) δ 1.23 and 1.33 (6H, 2s, $2\times2CH_3$), 2.75, 3.0~3.3 and 3.6~3.9 (11H, s and 2m, *N*-methylmorpholine), 3.40 (3H, s, OCH_3), 4.45 (1H, s, 3-H), 4.78 (1H, s, 2'-H), 5.18 (2H, s, OCH_2), 5.33 (1H, s, 5-H), 7.1~7.6 (10H, m, $2\times$Ph), 9.56 (1H, s, 6β-NH).

Anal Calcd for $C_{29}H_{37}N_3O_9S_2$: C 54.79, H 5.87, N 6.61, S 10.09.
Found: C 54.55, H 5.81, N 6.48, S 9.94.

Hydrogenation of **4a** in water - methanol (4 : 1) for 30 minutes, ion exchange on Amberlite IR-120 (Na^+) and freeze-drying afforded **6a** in 97 % yield. IR ν_{max} cm^{-1} 3425, 1760, 1678, 1605, 1208, 1098. ^1H NMR (90 MHz, DMSO-d_6) δ 1.34 (6H, s, $2\times2CH_3$), 3.42 (3H, s, OCH_3), 3.95 (1H, s, 3-H), 3.74 (1H, s, 2'-H), 5.32 (1H, s, 5-H), 7.1~7.7 (5H, m, Ph), 9.40 (1H, s, 6β-NH). FAB-MS *m/z* 489 (M + H, $C_{17}H_{19}N_2O_8S_2Na_2$).

Disodium 6α-Methoxy-[D,L-2-(4-methylphenyl)-2-sulfoacetamido]penicillanate (**6b**)

4b (M=Na) was prepared as described for **4a** followed by ion exchange on Amberlite IR-120 (Na^+) and freeze-drying, 28 % yield. IR ν_{max} ($CHCl_3$) cm^{-1} 3450, 1770, 1740, 1670, 1250, 1190, 1095. ^1H NMR (60 MHz) δ 1.28, 1.32 and 1.42 (6H, 3s, $2\times2CH_3$, epimers), 2.28 (3H, s, CH_3), 3.48 and 3.52 (3H, 2s, OCH_3, epimers), 4.48 and 4.53 (1H, 2s, 3-H, epimers), 4.93 and 5.18 (1H, 2s, 2'-H, epimers), 5.25 (2H, s, OCH_2), 5.51 (1H, s, 5-H), 7.09 and 7. 56 (4H, ABq, J=8 Hz, aromatic), 7.42 (5H, s, Ph), 9.18 and 9.22 (1H, 2s, 6β-NH, epimers).

4b was hydrogenated using the method described for **4a** with addition of an equivalent of $NaHCO_3$ to give **6b** in 85 % yield. IR ν_{max} cm^{-1} 3450, 1770, 1680, 1610, 1210, 1085. ^1H NMR (90 MHz, DMSO-d_6) δ 1.27 and 1.37 (6H, 2s, $2\times2CH_3$), 2.25 (3H, s, CH_3), 3.40 (3H, s, OCH_3), 3.95 and 4.04 (1H, 2s, 3-H, epimers), 4.45 and 4.78 (1H, 2s, 2'-H, epimers), 5.35 and 5.41 (1H, 2s, 5-H, epimers), 7.06 (2H, d, J=8 Hz, aromatic 3-H and 5-H), 7.39 and 7.40 (2H, 2d, J=8 Hz, aromatic 2-H and 6-H, epimers), 9.34 and 9.40 (1H, 2s, 6β-NH, epimers). FAB-MS *m/z* 503 (M+H, $C_{18}H_{21}N_2O_8S_2Na_2$).

Disodium 6β-[D,L-2-(4-Fluorophenyl)-2-sulfoacetamido]-6α-methoxypenicillanate (**6c**)

Compounds **4c** (M=Na) and **6c** were prepared using the procedure outlined for **4b** and **6b**.

4c: Yield 29 %. IR ν_{max} ($CHCl_3$) cm^{-1} 3470, 1775, 1745, 1685, 1245, 1095, 1042. ^1H NMR (60 MHz) δ 1.2~1.7 (6H, m, $2\times2CH_3$), 3.58 and 3.60 (3H, 2s, OCH_3, epimers), 4.53 and 4.60 (1H, 2s, 3-H, epimers), 4.80 and 5.13 (1H, 2s, 2'-H, epimers), 5.33 (2H, s, OCH_2), 5.57 (1H, s, 5-H), 7.0~8.0 (9H, m, aromatic), 9.43 and 9.60 (1H, 2s, 6β-NH, epimers).

6c: Yield 83%. IR ν_{max} cm^{-1} 3450, 1765, 1680, 1600, 1225, 1095. ^1H NMR (90 MHz, DMSO-d_6) δ 1.30 and 1.45 (6H, 2s, $2\times2CH_3$), 3.40 (3H, s, OCH_3), 3.89 and 3.95 (1H, 2s, 3-H, epimers), 4.46 and 4.78 (1H, s, 2'-H, epimers), 5.30 and 5.36 (1H, s, 5-H, epimers), 6.9~7.7 (4H, m, aromatic), 9.32 and 9.37 (1H, 2s, 6β-NH, epimers). FAB-MS *m/z* 507 (M+H, $C_{17}H_{18}FN_2O_8S_2Na_2$).

Disodium 6β-[D,L-2-(4-Hydroxyphenyl)-2-sulfoacetamido]-6α-methoxypenicillanate (**6d**)

Compounds **4d** (M=$HNEt_3$) and **6** (R=4-acetoxyphenyl) were prepared using the procedure out-

lined for **4b** and **6b**.

4d: Yield 30 %. IR ν_{max} cm^{-1} 3450, 1780, 1745, 1685, 1210, 1045, 1020. ^1H NMR (60 MHz) δ 1.0~1.7 (15H, m, $5\times CH_3$), 2.25 (3H, s, $COCH_3$), 3.07 (6H, q, J=7 Hz, triethylamine $3\times CH_2$), 3.57 (3H, s, OCH_3), 4.47 and 4.53 (1H, 2s, 3-H, epimers), 4.78 and 5.13 (1H, 2s, 2′-H, epimers), 5.23 (2H, s, OCH_2), 5.48 and 5.52 (1H, 2s, 5-H, epimers), 6.93 and 7.22 (4H, ABq, J=9 Hz, aromatic), 7.27 (5H, s, Ph), 9.17 and 9.33 (1H, 2s, 6β-NH, epimers).

6 (R=4-acetoxyphenyl): Yield 69%. IR ν_{max} cm^{-1} 3440, 1765, 1680, 1610, 1205, 1042, 1020. ^1H NMR (90 MHz, DMSO-d_6) δ 1.30 and 1.35 (3H, s, $2\times 2CH_3$), 1.23 (3H, s, $COCH_3$), 3.40 (3H, s, OCH_3), 3.89 and 3.96 (1H, s, 3-H, epimers), 4.44 and 4.76 (1H, 2s, 2′-H, epimers), 5.29 and 5.35 (1H, 2s, 5-H, epimers), 6.96 and 7.47 (4H, ABq, J=9 Hz, aromatic), 9.33 and 9.36 (1H, 2s, 6β-NH, epimers).

6 (R=4-acetoxyphenyl) (1.78 g) in water (10 ml) was stirred at room temperature for 2 hours with citrus acetylesterase (96 units) while pH 6.2 was maintained with 0.1 M sodium hydroxide. **6d** was isolated from the concentrated mixture by chromatography on Sephadex G-25 fine eluting with water and freeze-drying the fractions containing product, detected by HPLC, 1.63 g (99 %). IR ν_{max} cm^{-1} 3440, 1765, 1675, 1610, 1210, 1042. ^1H NMR (90 MHz, DMSO-d_6) δ 1.33 and 1.47 (6H, 2s, $2\times 2CH_3$), 3.37 and 3.40 (3H, 2s, OCH_3, epimers), 3.90 and 3.97 (1H, 2s, 3-H, epimers), 4.27 and 4.55 (1H, 2s, 2′-H, epimers), 5.29 and 5.35 (1H, 2s, 5-H, epimers), 6.64 (2H, d, J=9 Hz, aromatic 3-H and 5-H), 7.24 and 7.28 (2H, 2d, J=9 Hz, aromatic 2-H and 6-H, epimers), 9.32 (1H, s, 6β-NH). FAB-MS m/z 505 (M+H, $C_{17}H_{19}N_2O_9S_2Na_2$).

Disodium 6β-[D-2-(4-Aminophenyl)-2-sulfoacetamido]-6α-methoxypenicillanate (**6e**)

4e (M=$HNEt_3$), was prepared from 4-nitrophenylacetyl chloride using the procedure described for compound **4a** but with triethylamine in place of *N*-methylmorpholine. The unwanted epimer was eluted from the chromatography first, 8% yield. ^1H NMR (60 MHz) δ 1.28 (9H, t, J=7 Hz, triethylamine $3\times CH_3$), 1.45 and 1.60 (6H, 2s, $2\times 2CH_3$), 3.27 (6H, q, J=7 Hz, triethylamine $3\times CH_2$), 3.62 (3H, s, OCH_3), 4.67 (1H, s, 3-H), 5.13 (1H, s, 2′-H), 5.35 (2H, s, OCH_2), 5.63 (1H, s, 5-H), 7.56 (5H, s, Ph), 8.10 and 8.35 (4H, ABq, J=9 Hz, aromatic), 9.73 (1H, s, 6β-NH), followed by **4e**, 27% yield, which was crystallized from acetone - ether, mp 157~158°C. IR ν_{max} ($CHCl_3$) cm^{-1} 3300, 1780, 1740, 1675, 1515, 1460, 1345, 1035. ^1H NMR (90 MHz, DMSO-d_6) δ 1.18 (9H, t, J=7 Hz, triethylamine $3\times CH_3$), 1.23 and 1.36 (6H, 2s, $2\times 2CH_3$), 3.10 (6H, q, J=7 Hz, triethylamine $3\times CH_2$), 3.47 (3H, s, OCH_3), 4.50 (1H, s, 3-H), 5.10 (1H, s, 2′-H), 5.21 (2H, s, OCH_2), 5.36 (1H, s, 5-H), 7.39 (5H, s, Ph), 7.78 and 8.16 (4H, ABq, J=8 Hz, aromatic), 9.57 (1H, s, 6β-NH).

Anal Calcd for $C_{30}H_{40}N_4O_{10}S_2$: C 52.93, H 5.92, N 8.23, S 9.42.
Found: C 53.00, H 5.92, N 8.16, S 9.27.

6e was prepared from **4e** as described for **6a**, 87% yield. IR ν_{max} cm^{-1} 3450, 1765, 1675, 1610, 1205, 1040. ^1H NMR (90 MHz, D_2O) δ 1.28 and 1.43 (6H, 2s, $2\times 2CH_3$), 3.62 (3H, s, OCH_3), 4.26 (1H, s, 3-H), 5.07 (1H, s, 2′-H), 5.56 (1H, s, 5-H), 6.67 and 7.50 (4H, ABq, J=8.4 Hz, aromatic). FAB-MS m/z 504.0494 (M+H, calcd for $C_{17}H_{20}N_3O_8S_2Na_2$ 504.0488).

Disodium 6β-(D,L-2-Isoxazol-3-yl-2-sulfoacetamido)-6α-methoxypenicillanate (**6f**)

Compounds **4f** and **6f** were prepared using the methods described for **4b** and **6b**.

4f: Yield 6%. IR ν_{max} (CH_2Cl_2) cm^{-1} 1782, 1745, 1690, 1040. ^1H NMR (60 MHz) δ 1.1~1.6 (15H, m, $5\times CH_3$), 3.27 (6H, q, J=7 Hz, triethylamine $3\times CH_2$), 3.58 and 3.63 (3H, 2s, OCH_3, epimers), 4.58 (1H, s, 3-H), 5.16 and 5.48 (1H, 2s, 2′-H, epimers), 5.33 (2H, s, OCH_2), 5.55 (1H, s, 5-H), 6.88 and 8.77 (2H, 2d, J=2 Hz, isoxazole protons), 7.51 (5H, s, Ph), 9.38 and 9.48 (1H, 2s, 6β-NH, epimers).

6f: Yield 68%. IR ν_{max} cm^{-1} 3458, 1773, 1687, 1637, 1247, 1046. ^1H NMR (250 MHz, D_2O) δ 1.36, 1.40, 1.47 and 1.58 (6H, 4s, $2\times 2CH_3$, epimers), 3.45 and 3.59 (3H, 2s, OCH_3, epimers), 4.38 and 4.42 (1H, 2s, OCH_3, epimers), 4.38 and 4.42 (1H, 2s, 3-H), 5.39 and 5.42 (1H, 2s, 2′-H), 5.51 and 5.56 (1H, 2s, 5-H, epimers), 6.88 and 8.68 (2H, 2d, J=2 Hz, isoxazole protons). FAB-MS m/z 480 (M+H, $C_{14}H_{16}N_3O_9S_2Na_2$).

Disodium 6β-[D,L-2-(4-Bromophenyl)-2-sulfoacetamido]-6α-methoxypenicillanate (**6g**)

4g (M=Na) was prepared in 27 % yield as described for **4b**. IR ν_{max} ($CHCl_3$) cm^{-1} 3450, 1775, 1742, 1678, 1240, 1035. ^1H NMR (60 MHz) δ 1.27, 1.33 and 1.45 (6H, 3s, $2\times 2CH_3$, epimers), 3.50

and 3.55 (3H, 2s, OCH_3, epimers), 4.52 and 4.58 (1H, 2s, 3-H, epimers), 5.07 and 5.38 (1H, 2s, 2′-H, epimers), 5.24 (2H, s, OCH_2), 5.53 (1H, s, 5-H), 7.2~7.8 (9H, m, aromatic), 9.27 and 9.37 (1H, 2s, 6β-NH, epimers).

4g (0.5 g) in water (20 ml) was maintained at pH 10 with 1 M NaOH for 5 hours, adjusted to pH 5.5 with 5 M HCl and freeze-dried. **6g** was isolated by chromatography on silica gel eluting with BuOH - EtOH - H_2O, 4: 1: 1, 0.30 g (67 %). IR ν_{max} cm^{-1} 3450, 1764, 1680, 1605, 1208, 1041. ^1H NMR (90 MHz, D_2O) δ 1.12, 1.29 and 1.39 (6H, 3s, 2×$2CH_3$, epimers), 3.35 and 3.52 (3H, 2s, OCH_3, epimers), 4.15 and 4.23 (1H, 2s, 3-H, epimers), 5.07 (1H, s, 2′-H), 5.49 (1H, s, 5-H), 7.4~7.7 (4H, m, aromatic).

2-(2-Methylpropyloxysulfonyl)phenylacetic Acid (**19**)

2-Methylpropyl Phenylmethanesulfonate (**18**): Phenylmethanesulfonyl chloride (1.9 g) and pyridine (1 ml)] in 2-methylpropanol (15 ml) were heated under reflux for 30 minutes then evaporated. The residue in chloroform (50 ml) was washed with 1 M $NaHCO_3$, 1 M HCl and water, dried and evaporated to a low melting solid, mp 30~31°C, 0.98 g (43 %). IR ν_{max} (Nujol) cm^{-1} 1170, 980, 950, 780, 700. ^1H NMR (60 MHz, $CDCl_3$) δ 0.94 (6H, d, J=7.5 Hz, $CH(CH_3)_2$), 1.92 (1H, m, CH), 3.85 (2H, d, J=6.5 Hz, CH_2CH), 4.35 (2H, s, CH_2SO_3), 7.45 (5H, m, Ph).

19: A solution of **18** (4.44 g) in anhydrous THF (35 ml) was added dropwise to 2 M *n*-butyl lithium in hexane (12 ml) at −78°C. The orange solution was strirred at −70°C for 30 minutes, poured onto solid carbon dioxide and left to warm to room temperature. The mixture was concentrated, added ethyl acetate (50 ml) and extracted with 0.5 M $NaHCO_3$ (3×50 ml). The aqueous solution was acidified to pH 2.5 and extracted with ethyl acetate (3×50 ml). **19** was isolated as an oil on evaporation of the dried extracts, 3.33 g (63 %). IR ν_{max} (film) cm^{-1} 3540, 1740, 1360, 1175, 940. ^1H NMR (60 MHz, $CDCl_3$) δ 0.87 (6H, d, J=7.5 Hz, $CH(CH_3)_2$), 2.00 (1H, m, $CH(CH_3)_2$), 3.95 (2H, d, J=6.5 Hz, CH_2CH), 5.34 (1H, s, PhC*H*), 7.25~7.9 (5H, m, Ph), 10.36 (1H, br s, CO_2H). MS, on methyl ester formed with CH_2N_2, *m/z* 286.0858 (M^+, calcd for $C_{13}H_{15}O_5S$ 286.0873).

Disodium 6α-Methoxy-6β-(D,L-2-sulfo-2-thien-3-ylacetamido)penicillanate (**6h**)

Sodium Thien-3-ylmethanesulfonate: Thien-3-ylmethyl bromide (68.3 g) in ethanol (40 ml) was added to sodium sulfite heptahydrate (93 g) in water (200 ml) and stirred at 60°C for 4.5 hours and evaporated to dryness. The residue was treated with hot ethanol to provide the product in quantitative yield. IR ν_{max} (Nujol) cm^{-1} 1230, 1190, 1145. ^1H NMR (60 MHz, D_2O) δ 4.27 (2H, s, CH_2), 7.1~7.7 (3H, m, thienyl protons).

2-Methylpropyl Thien-3-ylmethanesulfonate: The sodium salt (0.64 g) in thionyl chloride (4 ml) containing DMF (16 μl) was stirred overnight, diluted with diisopropyl ether (20 ml), filtered and the filtrate evaporated to give the sulfonyl chloride. To this was added sequentially 2-methylpropanol (10 ml), triethylamine (0.28 ml) and 4-dimethylaminopyridine (24 mg). After 2 hours ether (75 ml) was added, the solution washed with 1 M HCl, 1 M $NaHCO_3$ and brine, dried, evaporated and the product isolated by chromatography on silica gel eluting with light petroleum (bp 60~80°C) - ethyl acetate, 10: 1, 0.26 g (59 %). IR ν_{max} (CH_2Cl_2) cm^{-1} 1675, 1350, 1170. ^1H NMR (60 MHz) δ 0.92 (6H, d, J=6 Hz, $CH(CH_3)_2$), 1.97 (1H, m, CH), 3.98 (2H, d, J=6 Hz, CH_2CH), 4.65 (2H, s, CH_2SO_3), 7.2~7.8 (3H, m, thienyl protons).

2-(2-Methylpropyloxysulfonyl)-2-thien-3-ylacetic acid was prepared in 90 % yield using the procedure described for **19**. IR ν_{max} (CH_2Cl_2) cm^{-1} 1720, 1360, 1170. ^1H NMR (60 MHz) δ 0.89 (6H, d, J=6 Hz, $CH(CH_3)_2$), 1.7~2.4 (1H, m, CH), 4.01 (2H, d, J=6 Hz, CH_2CH), 5.75 (1H, s, $CHSO_3$), 7.3~8.0 (3H, m, thienyl protons), 8.81 (1H, s, CO_2H).

Benzyl 6α-Methoxy-6β-[D,L-2-(2-methylpropyloxysulfonyl)-2-thien-3-ylacetamido]penicillanate (**4h**, M=$CH_2CH(CH_3)_2$): Prepared as described for compound **3e**, 52% yield. IR ν_{max} (CH_2Cl_2) cm^{-1} 3300, 1780, 1740, 1700, 1495, 1360, 1180. ^1H NMR (60 MHz) δ 0.81 (6H, d, J=6 Hz, $CH(CH_3)_2$), 1.28, 1.41, 1.55 (6H, 3s, 2×$2CH_3$, epimers), 2.07 (1H, m, CH), 3.35 and 3.59 (3H, 2s, OCH_3, epimers), 4.03 and 4.09 (2H, 2d, J=6 Hz, SO_3CH_2, epimers), 4.47 and 4.58 (1H, 2s, 3-H, epimers), 5.30 (2H, s, OCH_2), 5.60 and 5.66 (1H, 2s, 5-H, epimers), 5.93 (1H, s, 2′-H), 7.4~8.0 (8H, m, Ph and thienyl protons), 8.91 and 9.11 (1H, 2s, 6β-NH, epimers).

Sodium 6α-methoxy-6β-[D,L-2-(2-methylpropyloxysulfonyl)-2-thien-3-ylacetamido]penicillanate was prepared by hydrogenation of the benzyl ester as described for **3c**, 24% yield. IR ν_{max} (Nujol) cm^{-1} 1760, 1690, 1600, 1375, 1170. ^1H NMR (60 MHz, free acid) δ 0.93 (6H, d, J=7 Hz, $CH(CH_3)_2$), 1.33, 1.44, 1.55 and 1.59 (6H, 4s, 2×$2CH_3$, epimers), 2.0 (1H, m, CH), 3.36 and 3.59 (3H, 2s, OCH_3, epimers), 4.04 and 4.11 (2H, 2d, J=7 Hz, SO_3CH_2, epimers), 4.41 and 4.49 (1H, 2s, 3-H, epimers), 5.57 and 5.61 (1H, 2s, 5-H, epimers), 5.92 and 5.94 (1H, 2s, 2'-H, epimers), 6.2 (1H, br s, CO_2H), 7.4~8.0 (3H, m, thienyl protons), 8.86 and 9.06 (1H, 2s, 6β-NH, epimers).

6h: The sulfonate in water was maintained at pH 9 with $NaHCO_3$ for 29 hours, adjusted to pH 6 with Amberlite IR-120 (H^+) resin, filtered and freeze-dried to provide **6h**, quantitatively. IR ν_{max} (Nujol) cm^{-1} 1760, 1670, 1600. ^1H NMR (90 MHz, D_2O) δ 1.40, 1.53 and 1.61 (6H, 3s, 2×$2CH_3$, epimers), 3.59 and 3.76 (3H, 2s, OCH_3, epimers), 4.42 and 4.47 (1H, 2s, 3-H, epimers), 5.50 (1H, s, 5-H), 5.73 (1H, s, 2'-H), 7.4~7.8 (3H, m, thienyl protons).

Disodium 6α-Methoxy-6β-(D,L-2-sulfo-2-thien-2-ylacetamido)penicillanate (**6i**)

Sodium Thien-2-ylmethanesulfonate (**20**, M=Na): Prepared from thien-2-ylmethyl chloride as described for the 3-isomer, 20% yield. ^1H NMR (60 MHz, D_2O) δ 4.40 (2H, s, CH_2), 7.0~7.5 (3H, m, thienyl protons).

Ethyl Thien-2-ylmethanesulfonate: **20** (M=Na, 0.14 g) and cetyltrimethylammonium bromide (0.18 g) were shaken in water - dichloromethane, 1:1. The organic layer was evaporated to provide **20** (M=cetyltrimethylammonium). This, redissolved in dichloromethane (5 ml), was treated with triethyloxonium tetrafluoroborate (96 mg) in dichloromethane (0.4 ml). After 90 minutes the reaction was diluted with dichloromethane (20 ml), washed with water (2 × 10 ml), dried, evaporated and chromatographed on Fluorosil (10 g) eluting with ethyl acetate, 0.05 g (50 %). IR ν_{max} (CH_2Cl_2) cm^{-1} 1360, 1175. ^1H NMR (60 MHz, $CDCl_3$) δ 1.36 (3H, t, J=7 Hz, CH_3), 4.25 (2H, q, J=7 Hz, CH_2), 4.61 (2H, s, CH_2SO_3), 7.0~7.5 (3H, m, thienyl protons).

2-Ethoxysulfonyl-2-thien-2-ylacetic Acid: Prepared by the method described for compound **19**, 48% yield. IR ν_{max} (CH_2Cl_2) cm^{-1} 1720, 1360, 1180. ^1H NMR (60 MHz) δ 1.29 (3H, t, J=7 Hz, CH_3), 4.34 (2H, q, J=7 Hz, CH_2), 5.94 (1H, s, CH), 7.07~7.6 (3H, m, thienyl protons).

4i (M=CH_2CH_3): Prepared as described for compound **3c**, 48% yield. IR ν_{max} (CH_2Cl_2) cm^{-1} 3300, 1780, 1700, 1350, 1175. ^1H NMR (60 MHz) δ 1.0~1.5 (9H, m, 3×CH_3), 3.36 and 3.60 (3H, 2s, OCH_3, epimers), 4.2~4.5 (2H, m, OCH_2CH_3), 4.51 and 4.59 (1H, 2s, 3-H, epimers), 5.29 (2H, s, OCH_2), 5.60 (1H, s, 5-H), 6.08 (1H, s, 2'-H), 7.1~7.9 (8H, m, Ph and thienyl protons), 9.00 and 9.21 (1H, 2s, 6β-NH, epimers).

Sodium 6β-(D,L-2-Ethoxysulfonyl-2-thien-2-ylacetamido)-6α-methoxypenicillanate: Obtained by hydrogenation of **4i** (M=CH_2CH_3) using the method described for **3c**, 38% yield. ^1H NMR (60 MHz, free acid) δ 1.1~1.6 (9H, m, 3×CH_3), 3.36 and 3.60 (3H, 2s, OCH_3, epimers), 4.35 and 4.44 (2H, 2q, J=7 Hz, OCH_2CH_3, epimers), 4.40 (1H, s, 3-H), 5.54 (1H, s, 5-H), 6.09 (1H, s, 2'-H), 7.1~7.9 (3H, m, thienyl protons), 9.03 and 9.22 (1H, 2s, 6β-NH, epimers).

6i: The ethyl sulfonate (40 mg) was stirred in water for 5 hours, adjusted to pH 5.2 and evaporated to provide **6i**, 37 mg (93 %). IR ν_{max} (Nujol) cm^{-1} 1760, 1670, 1600. ^1H NMR (90 MHz, D_2O) δ 1.2~1.5 (6H, m, 2×$2CH_3$), 3.47 and 3.64 (3H, 2s, OCH_3, epimers), 4.40 and 4.44 (1H, 2s, 3-H, epimers), 5.56 and 5.63 (2H, 2s, 5-H and 2'-H), 7.1~7.7 (3H, m, thienyl protons).

Disodium 6α-Methylthio-6β-(D-2-phenyl-2-sulfoacetamido)penicillanate (**15a**)

Benzyl 6α-Methylthio-6β-(D-2-phenyl-2-sulfoacetamido)penicillanate *N*-Methylmorpholinium Salt (**9a**): Acylation of **7** with 2-phenyl-2-sulfoacetyl chloride by the method described for the preparation of **4a** provided **9** (M=*N*-methylmorpholinium) which was crystallized from acetone, yield 18%, mp 138~140°C. IR ν_{max} cm^{-1} 3440, 3250, 2760, 2645, 1775, 1750, 1670, 1455, 1303, 1170, 1032. ^1H NMR (90 MHz, DMSO-d_6) δ 1.23 and 1. 37 (6H, 2s, 2×$2CH_3$), 2.30 (3H, s, SCH_3), 2.77 and 3.0~3.9 (11H, s and m, *N*-methylmorpholine), 4.47 (1H, s, 3-H), 4.76 (1H, s, 2'-H), 5.18 (2H, s, OCH_2), 5.31 (1H, s, 5-H), 7.1~7.6 (10H, m, 2×Ph), 9.55 (1H, s, 6β-NH).

Anal Calcd for $C_{29}H_{37}N_3O_8S_3$: C 53.44, H 5.72, N 6.45, S 14.76.
Found: C 53.43, H 5.68, N 6.35, S 14.66.

Hydrogenation as described for **4a** provided **15a** in 62% yield. IR ν_{max} cm^{-1} 3460, 1760, 1670, 1605, 1403, 1210, 1043. ^1H NMR (90 MHz, DMSO-d_6) δ 1.34 (6H, 2s, 2×2CH_3), 2.27 (3H, s, SCH_3), 3.89 (1H, s, 3-H), 4.68 (1H, s, 2'-H), 5.26 (1H, s, 5-H), 7.1~7.6 (5H, m, Ph), 9.35 (1H, s, 6β-NH). FAB-MS *m/z* 505 (M+H, $C_{17}H_{19}N_2O_7S_3Na_2$).

General Procedure for the Preparation of **15b~g** *via* **12b~g**

To **9a** (1 mmol) in 1,2-dimethoxyethane or DMF (2 ml) was added X-H (1.1~10 mmol) and mercuric acetate (1 mmol). The mixture was stirred for 1 hour, diluted with ethyl acetate, washed with *N*-methylmorpholinium sulfide and filtered. The filtrate was washed with water (×3), dried, evaporated, chromatographed on sillica gel eluting with ethyl acetate - ethanol - water, 12: 2: 1 and ion exchanged to provide **12b~g** (M=Na). Hydrogenation of the benzyl ester and freeze-drying then gave **15b~g**.

12b: HX=H_2O, 10 mmol, yield 57%. **15b**: Yield 55%. IR ν_{max} cm^{-1} 3440, 1750, 1675, 1600, 1210, 1045. ^1H NMR (90 MHz, DMSO-d_6) δ 1.28 and 1.32 (6H, 2s, 2×2CH_3), 3.38 (br s, OH and H_2O), 3.85 (1H, s, 3-H), 4.54 (1H, s, 2'-H), 5.22 (1H, s, 5-H), 7.1~7.5 (5H, m, Ph), 9.33 (1H, s, 6β-NH). FAB-MS *m/z* 475 (M+H, $C_{16}H_{17}N_2O_8S_2Na_2$).

12c: HX=EtOH, 10 mmol, yield 45%. **15c**: Yield 70%. IR ν_{max} cm^{-1} 3447, 1763, 1683, 1610, 1248, 1210, 1044. ^1H NMR (250 MHz, DMSO-d_6) δ 1.12 (3H, t, *J*=7 Hz, OCH_2CH_3), 1.31 and 1.34 (6H, 2s, 2×2CH_3), 3.6~3.9 (2H, m, OCH_2), 3.92 (1H, s, 3-H), 4.68 (1H, s, 2'-H), 5.26 (1H, s, 5-H), 7.2~7.5 (5H, s, Ph), 9.37 (1H, s, 6β-NH). FAB-MS *m/z* 503 (M+H, $C_{18}H_{21}N_2O_8S_2Na_2$).

12d: HX=CH_3NH_2, 1.1 mmol, yield 72%. **15d**: Yield 86%. IR ν_{max} cm^{-1} 3440, 1765, 1665, 1605, 1214, 1044. ^1H NMR (90 MHz, DMSO-d_6) δ 1.34 (6H, s, 2×2CH_3), 2.32 (3H, s, NCH_3), 4.03 (1H, s, 3-H), 4.67 (1H, s, 2'-H), 5.16 (1H, s, 5-H), 7.1~7.6 (5H, m, Ph), 9.12 (1H, s, 6β-NH). FAB-MS *m/z* 488 (M+H, $C_{17}H_{20}N_3O_7S_2Na_2$).

12e: HX=$(CH_3)_2NH$, 1.1 mmol, yield 70%. **15e**: Yield 81%. IR ν_{max} cm^{-1} 3440, 1760, 1672, 1605, 1210, 1043. ^1H NMR (90 MHz, DMSO-d_6) δ 1.36 (6H, s, 2×2CH_3), 2.30 (6H, s, $N(CH_3)_2$), 3.98 (1H, s, 3-H), 4.62 (1H, s, 2'-H), 5.30 (1H, s, 5-H), 7.1~7.6 (5H, s, Ph), 8.97 (1H, s, 6β-NH). FAB-MS *m/z* 490 (M+H, $C_{18}H_{22}N_3O_7S_2Na_2$).

12f: HX=$CH_3NHOH \cdot HCl$, 1.1 mmol, with NEt_3, 1.1 mmol, yield 87%. **15f**: Yield 58%. IR ν_{max} cm^{-1} 3430, 1765, 1675, 1605, 1215, 1045. ^1H NMR (90 MHz, DMSO-d_6) δ 1.36 (6H, s, 2×2CH_3), 2.64 (3H, s, NCH_3), 3.97 (1H, s, 3-H), 4.65 (1H, s, 2'-H), 5.49 (1H, s, 5-H), 7.0~7.6 (5H, m, Ph), 8.48 (1H, br s, OH), 9.13 (1H, s, 6β-NH). FAB-MS *m/z* 504 (M+H, $C_{17}H_{20}N_3O_8S_2Na_2$).

12g: HX=$CH_3ONH_2 \cdot HCl$, 1.1 mmol, with NEt_3, 1.1 mmol, yield 62%. **15g**: Yield 65%. IR ν_{max} cm^{-1} 3440, 1760, 1670, 1605, 1214, 1048. ^1H NMR (90 MHz, DMSO-d_6) δ 1.24 and 1.32 (6H, 2s, 2×2CH_3), 3.45 (3H, s, OCH_3), 3.95 (1H, s, 3-H), 4.65 (1H, s, 2'-H), 5.32 (1H, s, 5-H), 7.0~7.6 (6H, m, Ph and 6α-NH), 9.30 (1H, s, 6β-NH). FAB-MS *m/z* 504 (M+H, $C_{17}H_{20}N_3O_8S_2Na_2$).

Sodium 6β-[D-2-(2,3-Dioxo-4-ethylpiperazin-1-ylcarbonylamino)-2-phenylacetamido]-6α-methoxypenicillanate (**17a**)

Benzyl 6α-Methylthio-6β-[D-2-(4-nitrobenzyloxycarbonylamino)-2-phenylacetamido]penicillanate (**10**, R=Ph): *N*-4-Nitrobenzyloxycarbonyl-D-phenylglycine (10.1 g) was converted to its acid chloride using oxalyl chloride (3.6 ml) and this used to acylate **7** by the procedure described for compound **3e**, 15.74 g (yield 93%), crystallized from ethyl acetate - cyclohexane, mp 88~91°C. IR ν_{max} (CH_2Cl_2) cm^{-1} 3310, 1782, 1745, 1680, 1525, 1270. ^1H NMR (60 MHz, $CDCl_3$) δ 0.88 and 1.67 (6H, 2s, 2×2CH_3), 2.25 (3H, s, SCH_3), 4.33 (1H, s, 3-H), 5.17 and 5.28 (4H, 2s, 2×OCH_2), 5.58 (1H, s, 5-H), 5.77 (1H, d, *J*=8 Hz, 2'-H), 6.70 (1H, d, *J*=8 Hz, 2'-NH), 7.0~8.3 (10H, m, aromatic and 6β-NH).

Anal Calcd for $C_{32}H_{32}N_4O_8S_2$: C 57.82, H 4.85, N 8.42, S 9.65.
Found: C 58.09, H 4.83, N 8.12, S 9.52.

Benzyl 6α-Methoxy-6β-[D-2-(4-nitrobenzyloxycarbonylamino)-2-phenylacetamido]penicillanate (**13a**): **10** (R=Ph, 4.05 g) and mercuric acetate (1.95 g) in DMF (7 ml) and methanol (100 ml) were stirred for 2 hours, concentrated, diluted with ethyl acetate (100 ml), washed with water (4×50 ml), dried, evaporated and the residue crystallized from toluene and recrystallized from ethyl acetate - cyclohexane, mp 88~90°C, 2.83 g (72%). ^1H NMR (60 MHz, $CDCl_3$) δ 0.83 and 1.20 (6H, 2s, 2×

$2CH_3$), 3.52 (3H, s, OCH_3), 4.37 (1H, s, 3-H), 5.23 and 5.30 (4H, 2s, $2\times OCH_2$), 5.67 (1H, s, 5-H), 5.68 (1H, d, J=7 Hz, 2'-H), 6.55 (1H, d, J=7 Hz, 2'-NH), 7.1~8.4 (10H, m, aromatic and 6β-NH).

Anal Calcd for $C_{32}H_{32}N_4O_6S$: C 59.26, H 4.94, N 8.64, S 4.94.
Found: C 59.35, H 5.11, N 8.33, S 4.84.

6β-(D-2-Amino-2-phenylacetamido)-6α-methoxypenicillanate (**16a**): **13a** (2.7 g) was hydrogenated in ethanol - water, 12: 1, (130 ml) for 30 minutes, filtered, concentrated then diluted with water (10 ml). The aqueous solution was washed well with $CHCl_3$ and diluted with propan-2-ol to give **16a** (X=OCH_3) as a colourless solid, 1.26 g (86%). IR ν_{max} cm^{-1} 3420, 1745, 1700, 1620, 1350, 1098. ^1H NMR (90 MHz, DMSO-d_6) δ 0.87 and 1.20 (6H, 2s, $2\times 2CH_3$), 3.34 (3H, s, OCH_3), 3.96 (1H, s, 3-H), 4.76 (1H, s, 2'-H), 4.97 (br s, NH_3^+, H_2O), 5.24 (1H, s, 5-H), 7.1~7.6 (6H, m, aromatic and 6β-NH). FAB-MS m/z 380 (M+H, $C_{17}H_{22}N_3O_5S$).

17a: 2,3-Dioxo-4-ethylpiperazin-1-ylcarbonyl chloride (**21**) (0.3 g) in THF (7.5 ml) was added dropwise to a cooled solution of **16a** (X=OCH_3) (0.6 g) in water (15 ml) and THF (7.5 ml) while pH 7.5±0.5 was maintained by the addition of triethylamine. After 30 minutes the solution was washed with ether, acidified to pH 2 and extracted with ethyl acetate (3×15 ml). The extracts were washed with water and brine, dried and evaporated to a foam, 0.76 g. This was dissolved in acetone treated with 1.85 M sodium 2-ethylhexanoate in 4-methylpentan-2-one (0.58 ml) and diluted with ether to precipitate the product, 0.69 g (69%). IR ν_{max} cm^{-1} 3400, 3245, 1762, 1712, 1675, 1610, 1510, 1187, 1095. ^1H NMR (90 MHz, DMSO-d_6) δ 0.83 and 1.21 (6H, 2s, $2\times 2CH_3$), 1.18 (3H, t, J=7 Hz, CH_2CH_3), 3.37 (3H, s, OCH_3), 3.2~4.0 (6H, m, $3\times CH_2$), 3.79 (1H, s, 3-H), 5.30 (1H, s, 5-H), 5.57 (1H, d, J=7 Hz, 2'-H), 7.2~7.6 (5H, m, Ph), 9.70 (1H, s, 6β-NH), 9.76 (1H, d, J=7 Hz, 2'-NH). FAB-MS m/z 570 (M+H, $C_{24}H_{29}N_5O_8SNa$).

Sodium 6β-[D-2-(2,3-Dioxo-4-ethylpiperazin-1-ylcarbonylamino)-2-phenylacetamido]-6α-hydroxypenicillanate (**17f**)

Compound **17f** was prepared from **10** (R=Ph) with water in 1,2-dimethoxyethane *via* **13f** and **16f** using the procedures described for the preparations of **13a**, **16a** and **17a**.

13f: Yield 53%. IR ν_{max} cm^{-1} 3300, 1775, 1740, 1680, 1517, 1347. ^1H NMR (60 MHz, $CDCl_3$) δ 0.92 and 1.26 (6H, 2s, $2\times 2CH_3$), 4.40 (1H, s, 3-H), 5.19 (4H, s, $2\times OCH_2$), 5.60 (1H, s, 5-H), 5.67 (1H, d, J=8 Hz, 2'-H), 6.18 (1H, br s, OH), 6.90 (1H, d, J=8 Hz, 2'-NH), 7.1~8.3 (9H, m, aromatic), 8.60 (1H, s, 6β-NH).

16f: Yield 52%. IR ν_{max} cm^{-1} 3400, 3200, 1760, 1600, 1240, 1120. ^1H NMR (90 MHz, DMSO-d_6) δ 0.89 and 1.22 (6H, 2s, $2\times 2CH_3$), 4.04 (1H, s, 3-H), 4.4 (br s, NH_3^+, OH, H_2O), 4.71 (1H, s, 2'-H), 5.25 (1H, s, 5-H), 7.1~7.7 (6H, m, Ph and 6β-NH). FAB-MS m/z 366 (M+H, $C_{16}H_{20}N_3O_5S$).

17f: Yield 42%. IR ν_{max} cm^{-1} 3400, 1762, 1710, 1675, 1605, 1510, 1368, 1185. ^1H NMR (90 MHz, DMSO-d_6) δ 0.83 and 1.20 (6H, 2s, $2\times 2CH_3$), 1.08 (3H, t, J=7 Hz, CH_2CH_3), 3.36 (2H, q, J=7 Hz, CH_2CH_3), 3.4~4.0 (4H, m, $2\times CH_2$), 3.77 (1H, s, 3-H), 5.28 (1H, s, 5-H), 5.56 (1H, d, J=8 Hz, 2'-H), 7.2~7.6 (5H, m, Ph), 9.53 (1H, s, 6β-NH), 9.86 (1H, d, J=8H, 2'-NH). FAB-MS m/z 556 (M+H, $C_{23}H_{27}N_5O_8SNa$).

Sodium 6β-[D-2-(2,3-Dioxo-4-ethylpiperazin-1-ylcarbonylamino)-2-phenylacetamido]-6α-ethoxypenicillanate (**17g**)

Prepared from **10** (R=Ph) with ethanol in 1,2-dimethoxyethane *via* **13g** and **16g** using the procedure described for **13a**, **16a** and **17a**.

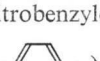
13g: Yield 51%. IR ν_{max} cm^{-1} 3300, 1773, 1740, 1675, 1520, 1348. ^1H NMR (60 MHz, $CDCl_3$) δ 0.91 and 1.27 (6H, 2s, $2\times 2CH_3$), 1.27 (3H, t, J=7 Hz, CH_2CH_3), 3.80 (2H, q, J=7 Hz, CH_2CH_3), 4.40 (1H, s, 3-H), 5.23 (4H, s, $2\times OCH_2$), 5.63 (1H, d, J=8 Hz, 2'-H), 5.65 (1H. s 5-H), 6.83 (1H, d, J=8 Hz, 2'-NH), 7.0~8.3 (9H, m, aromatic), 8.95 (1H, s, 6β-NH).

16g: Yield 65%. IR ν_{max} cm^{-1} 3400, 3260, 1752, 1600, 1345, 1092. ^1H NMR (90 MHz, DMSO-d_6) δ 0.94 and 1.24 (6H, 2s, $2\times 2CH_3$), 1.13 (3H, t, J=7 Hz, CH_2CH_3), 3.65 (2H, q, J=7 Hz, CH_2CH_3), 4.02 (1H, s, 3-H), 4.35 (br s, NH_3^+, H_2O), 4.74 (1H, s, 2'-H), 5.26 (1H, s, 5-H), 7.1~7.7 (6H, m, Ph, 6β-NH).

17g: Yield 78%. IR ν_{max} cm^{-1} 3400, 3290, 1760, 1710, 1672, 1610, 1510, 1400, 1368, 1190, 1095. ^1H NMR (90 MHz, DMSO-d_6) δ 0.83 and 1.20 (6H, 2s, $2\times 2CH_3$), 1.09 and 1.13 (6H, 2t, J=7 Hz, $2\times$

CH_2CH_3), 3.2~4.1 (8H, m, 4×CH_2), 3.80 (1H, s, 3-H), 5.32 (1H, s, 5-H), 5.61 (1H, d, J=8 Hz, 2-H), 7.2~7.7 (5H, m, Ph), 9.68 (1H, s, 6β-NH), 9.83 (1H, d, J=8 Hz, 2'-NH). FAB-MS m/z 584 (M+H, $C_{25}H_{31}N_5O_5SNa$).

Sodium 6β-[D-2-(2,3-Dioxo-4-ethylpiperazin-1-ylcarbonylamino)-2-(4-hydroxyphenyl)acetamido]-6α-methoxypenicillanate (**17b**)

N-4-Nitrobenzyloxycarbonyl-D-(4-benzyloxycarbonyloxyphenyl)glycine was elaborated through **10** (R= zo- ), **13b** and **16b** to **17b** as described for the preparation of **17a** above.

10 (R= zo- ): Yield 53%, mp 103~105°C. IR ν_{max} cm^{-1} 3300, 1760, 1670, 1608, 1520, 1348, 1220, 1055. ^1H NMR (60 MHz, $CDCl_3$) δ 0.90 and 1.18 (6H, 2s, 2×$2CH_3$), 2.27 (3H, s, SCH_3), 4.33 (1H, s, 3-H), 5.17, 5.25 and 5.30 (6H, 3s, 3×OCH_2), 5.57 (1H, s, 5-H), 5.67 (1H, d, J=7 Hz, 2'-H), 6.48 (1H, d, J=7 Hz, 2'-NH), 6.9~8.3 (19H, m, aromatic and 6β-NH).

Anal Calcd for $C_{40}H_{38}N_4O_{11}S_2$: C 58.97, H 4.67, N 6.88, S 7.86.
Found: C 59.03, H 4.99, N 6.60, S 7.88.

13b: Yield 90%, mp 107~109°C. IR ν_{max} cm^{-1} 3410, 3305, 1770, 1690, 1610, 1500, 1350, 1240. ^1H NMR (60 MHz, $CDCl_3$) δ 0.88 and 1.19 (6H, 2s, 2×$2CH_3$), 3.43 (3H, s, OCH_3), 4.29 (1H, s, 3-H), 5.13 (4H, s, 2×OCH_2), 5.21 (2H, s, OCH_2), 5.45 (1H, d, J=8 Hz, 2'-H), 5.56 (1H, s, 5-H), 6.30 (1H, d, J=8 Hz, 2'-NH), 7.0~8.3 (19H, m, aromatic and 6β-NH).

16b: Yield 74%. IR ν_{max} cm^{-1} 3400, 3200, 1760, 1690, 1610, 1515, 1255, 1100. ^1H NMR (90 MHz, DMSO-d_6) δ 0.95 and 1.28 (6H, 2s, 2×$2CH_3$), 3.39 (3H, s, OCH_3), 4.02 (1H, s, 3-H), 4.81 (1H, s, 2'-H), 5.31 (1H, s, 5-H), 6.4~8.0 (br s, NH_3^+, OH, H_2O), 6.75 and 7.35 (4H, ABq, J=9 Hz, aromatic), 9.31 (1H, s, 6β-NH). FAB-MS m/z 396 (M+H, $C_{17}H_{22}N_3O_6S$).

17b: Yield 49%. IR ν_{max} cm^{-1} 3400, 1764, 1710, 1680, 1610, 1510, 1368, 1195, 1095. ^1H NMR (90 MHz, DMSO-d_6) δ 0.93 and 1.26 (6H, 2s, 2×$2CH_3$), 1.08 (3H, t, J=7 Hz, CH_2CH_3), 3.36 (3H, s, OCH_3), 3.1~4.1 (6H, m, 3×CH_2), 3.85 (1H, s, 3-H), 5.30 (1H, s, 5-H), 5.45 (1H, d, J=8 Hz, 2'-H), 6.70 and 7.24 (4H, ABq, J=9 Hz, aromatic), 9.52 (1H, s, 6β-NH), 9.60 (1H, d, J=8 Hz, 2'-NH). FAB-MS m/z 586 (M+H, $C_{24}H_{29}N_5O_9SNa$).

2- and 3-Isomers of Sodium 6β-[D-2-(2,3-Dioxo-4-ethylpiperazin-1-yl-carbonylamino)-2-thienyl-acetamido]-6α-methoxypenicillanate (**17c** and **17d**)

2- and 3-*N*-(4-Nitrobenzyloxycarbonyl)-D,L-thienylglycines were coupled to **7** and the products **10** (R=2- and 3-thienyl), epimeric at 2', treated with methanol and mercuric acetate as described for **13a**. The epimers of both compounds, **13c** and **13d**, were separated by chromatography on silica gel eluting with ethyl acetate - cyclohexane, 1:4. Each was treated by the procedures described for the preparation of **16a** and **17a** to give **16c** and **16d** then **17c** and **17d**.

10 (R=2-thienyl): Yield 77%. IR ν_{max} cm^{-1} 3300, 1780, 1740, 1680, 1520, 1348, 1230, 1205. ^1H NMR (60 MHz, $CDCl_3$) δ 1.10, 1.25, 1.30 and 1.35 (6H, 4s, 2×$2CH_3$, epimers), 2.02 and 2.20 (3H, 2s, SCH_3, epimers), 4.34 and 4.40 (1H, 2s, 3-H, epimers), 5.15, 5.16 and 5.22 (4H, 3s, 2×OCH_2, epimers), 5.52 (1H, s, 5-H), 5.80 and 5.88 (1H, 2d, J=8 Hz, 2'-H, epimers), 6.31 (1H, d, J=8 Hz, 2'-NH), 6.8~7.5 (8H, m, thienyl protons and Ph), 7.47 and 8.15 (4H, ABq, J=9 Hz, aromatic), 7.75 (1H, s, 6β-NH). MS m/z 670.1194 (M^+, calcd for $C_{30}H_{29}N_4O_9S_3$ 670.1178).

13c: D-Stereoisomer, 32%. IR ν_{max} cm^{-1} 3310, 1775, 1743, 1685, 1520, 1348, 1260, 1207. ^1H NMR (60 MHz, $CDCl_3$) δ 1.08 and 1.25 (6H, 2s, 2×$2CH_3$), 3.42 (3H, s, OCH_3), 4.35 (1H, s, 3-H), 5.15 and 5.22 (4H, 2s, 2×OCH_2), 5.59 (1H, s, 5-H), 5.83 (1H, d, J=8 Hz, 2'-H), 6.29 (1H, d, J=8 Hz, 2'-NH), 6.8~7.4 (8H, m, thienyl protons and Ph), 7.45 and 8.15 (4H, ABq, J=9 Hz, aromatic), 7.75 (1H, s, 6β-NH). MS m/z 654.1405 (M^+, calcd for $C_{30}H_{29}N_4O_9S_2$ 654.1409).

16c: Yield 48%. IR ν_{max} cm^{-1} 3420, 3280, 1760, 1605, 1345, 1250, 1095. ^1H NMR (90 MHz, DMSO-d_6) δ 1.15 and 1.31 (6H, 2s, 2×$2CH_3$), 3.37 (3H, s, OCH_3), 4.10 (1H, s, 3-H), 4.78 (br s, NH_3^+, H_2O), 4.97 (1H, s, 2'-H), 5.33 (1H, s, 5-H), 6.9~7.5 (4H, m, thienyl protons and 6β-NH).

17c: Yield 52%. IR ν_{max} cm^{-1} 3420, 3300, 1763, 1710, 1680, 1610, 1520, 1400, 1368, 1190, 1095.

^{1}H NMR (90 MHz, DMSO-d_6) δ 1.05 and 1.29 (6H, 2s, 2×2CH$_3$), 1.08 (3H, t, J=7 Hz, CH$_2$CH_3), 3.1~4.1 (6H, m, 3×CH$_2$), 3.83 (1H, s, 3-H), 5.33 (1H, s, 5-H), 5.88 (1H, d, J=8 Hz, 2'-H), 6.9~7.5 (3H, m, thienyl protons), 9.71 (1H, d, J=8 Hz, 2'-NH), 9.79 (1H, s, 6β-NH).

10 (R=3-thienyl): Yield 63%. ^{1}H NMR (60 MHz, CDCl$_3$) δ 1.06, 1.23 and 1.33 (6H, 3s, 2×2CH$_3$, epimers), 2.03 and 2.22 (3H, 2s, SCH$_3$, epimers), 4.40 and 4.45 (1H, 2s, 3-H, epimers), 5.20 and 5.27 (4H, 2s, 2×OCH$_2$), 5.60 (1H, s, 5-H), 5.87 and 5.93 (1H, 2d, J=8 Hz, 2'-H, epimers), 6.77 (1H, d, J=8 Hz, 2'-NH), 6.9~8.3 (13H, m, 6β-NH, aromatic and thienyl protons).

13d: D-Epimer, yield 41%. ^{1}H NMR (60 MHz, CDCl$_3$) δ 1.02 and 1.25 (6H, 2s, 2×2CH$_3$), 3.50 (3H, s, OCH$_3$), 4.43 (1H, s, 3-H), 5.25 and 5.33 (4H, 2s, 2×OCH$_2$), 5.72 (1H, s, 5-H), 5.93 (1H, d, J=8 Hz, 2'-H), 6.65 (1H, d, J=8 Hz, 2'-NH), 7.0~8.4 (13H, m, 6β-NH, aromatic and thienyl protons).

16d: Yield 63%. IR ν_{max} cm^{-1} 3410, 1770, 1695, 1600, 1510, 1242, 1090. ^{1}H NMR (90 MHz, CD$_3$OD) δ 1.05 and 1.38 (6H, 2s, 2×2CH$_3$), 3.61 (3H, s, OCH$_3$), 4.21 (1H, s, 3-H), 5.22 (1H, s, 2'-H), 5.51 (1H, s, 5-H), 7.2~7.8 (3H, m, thienyl protons).

17d: Yield 33%. IR ν_{max} cm^{-1} 3430, 3320, 1765, 1712, 1675, 1605, 1510, 1190, 1095. ^{1}H NMR (250 MHz, DMSO-d_6) δ 1.02 and 1.28 (6H, 2s, 2×2CH$_3$), 1.08 (3H, t, J=7 Hz, CH$_2$CH_3), 3.38 (3H, s, OCH$_3$), 3.40 (2H, q, J=7 Hz, CH_2CH$_3$), 3.5~3.6 (2H, m, CH$_2$), 3.82 (1H, s, 3-H), 3.85~3.95 (1H, m, CH$_2$), 5.30 (1H, s, 5-H), 5.69 (1H, d, J=8 Hz, 2'-H), 7.1~7.6 (3H, m, thienyl protons), 9.68 (1H, d, J=8 Hz, 2'-NH), 9.75 (1H, s, 6β-NH).

Sodium 6β-[D,L-2-(2,3-Dioxo-4-ethylpiperazin-1-ylcarbonylamino)-2-fur-2-ylacetamido]-6α-methoxypenicillanate (**17e**)

Benzyl 6β-(D,L-2-Benzyloxycarbonylamino-2-fur-2-ylacetamido)-6α-methylthiopenicillanate (**10**, R=2-furyl): *N*-Benzyloxycarbonyl-2-fur-2-ylglycine (1.38 g) in dichloromethane (15 ml) was added dropwise to a solution of **7** (1.94 g) and DCC (1.13 g) in dichloromethane (5 ml) at 0°C. The mixture was stirred overnight, filtered and the product isolated from the filtrate by chromatography on silica gel eluting with ethyl acetate - cyclohexane, 1: 4, 2.37 g (78%). IR ν_{max} (CHCl$_3$) cm^{-1} 3320, 1780, 1742, 1690, 1310. ^{1}H NMR (60 MHz, CDCl$_3$) δ 1.20, 1.25, 1.30 and 1.37 (6H, 4s, 2×2CH$_3$, epimers), 2.07 and 2.21 (3H, 2s, SCH$_3$, epimers), 4.35 and 4.40 (1H, 2s, 3-H, epimers), 5.10 and 5.15 (4H, 2s, 2×OCH$_2$), 5.49 (1H, s, 5-H), 5.67 and 5.77 (1H, 2d, J=8 Hz, 2'-H), 6.0~6.4 (3H, m, 2'-NH, furyl 3-H and 4-H), 7.1~7.4 (11H, m, 2×Ph and furyl 5-H), 7.28 and 7.98 (1H, 2s, 6β-NH, epimers).

13e, **16e** and **17e** were prepared as mixtures of epimers by the procedures described for **13a**, **16a** and **17a**.

13e: Yield 83%. ^{1}H NMR (60 MHz, CDCl$_3$) δ 1.17, 1.25, 1.30 and 1.37 (6H, 4s, 2×2CH$_3$, epimers), 3.30 and 3.43 (3H, 2s, OCH$_3$, epimers), 4.40 and 4.43 (1H, 2s, 3-H, epimers), 5.13 and 5.16 (4H, 2s, 2×OCH$_2$), 5.60 (1H, s, 5-H), 5.70 and 5.80 (1H, 2d, J=8 Hz, 2'-H, epimers), 6.1~6.4 (3H, m, 2'-NH, furyl 3-H and 4-H), 7.1~7.4 (11H, m, 2×Ph and furyl 5-H), 8.00 and 8.25 (1H, 2s, 6β-NH, epimers).

16e: Isolated by freeze-drying of the aqueous solution, yield 78%. IR ν_{max} cm^{-1} 3405, 1765, 1705, 1600, 1330, 1250, 1092. ^{1}H NMR (90 MHz, D$_2$O) δ 1.20, 1.37, 1.42 and 1.49 (6H, 4s, 2×2CH$_3$, epimers), 3.40 and 3.55 (3H, 2s, OCH$_3$, epimers), 4.25 and 4.30 (1H, 2s, 3-H, epimers), 5.45 and 5.52 (1H, 2s, 2'-H, epimers), 5.57 and 5.59 (1H, 2s, 5-H, epimers), 6.5~6.9 and 7.6~7.8 (3H, m, furyl protons).

17e: Yield 52%. IR ν_{max} cm^{-1} 3400, 3300, 1765, 1710, 1680, 1610, 1505, 1190, 1095. ^{1}H NMR (90 MHz, DMSO-d_6) δ 1.08 (3H, t, J=7 Hz, CH$_2$CH_3), 1.12, 1.31 and 1.38 (6H, 3s, 2×2CH$_3$, epimers), 3.15 and 3.34 (3H, 2s, OCH$_3$, epimers), 3.0~4.1 (6H, m, 3×CH$_2$), 3.88 and 3.91 (1H, 2s, 3-H, epimers), 5.31 (1H, s, 5-H), 5.76 (1H, d, J=8 Hz, 2'-H), 6.3~6.6 and 7.5~7.7 (3H, m, furyl protons), 9.5~9.8 (2H, m, 2×NH). FAB-MS m/z 560 (M+H, C$_{22}$H$_{27}$N$_5$O$_9$SNa).

Sodium 6β-[D-2-(2,3-Dioxo-4-ethylpiperazin-1-ylcarbonylamino)-2-phenylacetamido]-6α-methylthiopenicillanate (**17h**)

D-*N*-(2,3-Dioxo-4-ethylpiperazin-1-ylcarbonyl)-2-phenylglycine (**8**, R=Ph, Y=NH-pip): D-2-Phenylglycine (8.4 g) in water (100 ml) and acetone (20 ml) was maintained at pH 9.5 with 2.5 M NaOH while **21** (10.25 g) in acetone (40 ml) was added dropwise. The solution was stirred for 30 minutes, washed with ether (2×100 ml), acidified to pH 2 and extracted with ethyl acetate (3×50 ml). The ethyl acetate solution was washed with water (2×50 ml) and brine (50 ml), dried and evaporated to

provide a foam, 13.8 g (86%). ^{1}H NMR (60 MHz) δ 1.13 (3H, t, J=8 Hz, CH_2CH_3), 3.48 (2H, q, J=8 Hz, CH_2CH_3), 3.5~4.2 (4H, m, 2×CH_2), 5.53 (1H, d, J=7 Hz, CH), 6.72 (1H, br s, CO_2H), 7.47 (5H, s, Ph), 9.98 (1H, d, J=7 Hz, NH).

11: The side chain acid above (13.8 g) was used to prepare compound **11** by the procedure described for **10** (R=Ph). Chromatography of the crude product on silica gel eluting with methyl acetate - cyclohexane, 1: 1, provided a small amount of the L-stereoisomer, 0.48 g, followed by the D-stereoisomer, which crystallized from methanol, mp 216~217°C, 9.77 g (35%). IR ν_{max} ($CHCl_3$) cm^{-1} 3300, 1780, 1748, 1720, 1695, 1182. ^{1}H NMR (90 MHz, DMSO-d_6) δ 0.92 and 1.11 (6H, 2s, 2×2CH_3), 1.07 (3H, t, J=7.5 Hz, CH_2CH_3), 2.35 (3H, s, SCH_3), 3.37 (2H, q, J=7.5 Hz, CH_2CH_3), 3.4~4.0 (4H, m, 2×CH_2), 4.45 (1H, s, 3-H), 5.15 (2H, s, OCH_2), 5.30 (1H, s, 5-H), 5.56 (1H, d, J=7 Hz, 2′-H), 7.35 (10H, s, 2×Ph), 9.28 (1H, d, J=7 Hz, 2′-NH), 9.88 (1H, s, 6β-NH).

Anal Calcd for $C_{31}H_{35}N_5O_7S_2$: C 56.95, H 5.40, N 10.71, S 9.81.
Found: C 56.82, H 5.35, N 10.80, S 9.72.

17h: Produced by hydrogenation of **11** in THF and isolation of the product as its sodium salt, yield 34%. IR ν_{max} cm^{-1} 3420, 1763, 1715, 1670, 1605, 1500, 1187. ^{1}H NMR (90 MHz, DMSO-d_6) δ 0.89 and 1.21 (6H, 2s, 2×2CH_3), 1.08 (3H, t, J=7.5 Hz, CH_2CH_3), 2.23 (3H, s, SCH_3), 3.38 (2H, q, J=7.5 Hz, CH_2CH_3), 3.4~4.4 (4H, m, 2×CH_2), 3.78 (1H, s, 3-H), 5.27 (1H, s, 5-H), 5.58 (1H, d, J=7 Hz, 2′-H), 7.1~7.6 (5H, m, Ph), 9.72 (1H, s, 6β-NH), 9.83 (1H, d, J=7 Hz, 2′-NH). FAB-MS m/z 586 (M+H, $C_{24}H_{29}N_5O_7S_2Na$).

General Procedure for the Preparation of **17i~l** *via* **14i~l**

To **11** (1 mmol) in DMF (2 ml) was added X-H (1.1 mmol) and mercuric acetate (1 mmol). After 30 minutes, ethyl acetate (50 ml) was added, the mixture filtered and the filtrate washed with water (3× 25 ml) and brine (25 ml), dried, evaporated and chromatogrphed to give **14i~l**. Hydrogenation in THF provided **17i~l** isolated as their sodium salts.

14i: HX=CH_3NH_2, yield 58%. **17i**: Yield 96%. IR ν_{max} cm^{-1} 3410, 3300, 1765, 1715, 1670, 1605, 1368, 1187. ^{1}H NMR (90 MHz, DMSO-d_6) δ 0.94 and 1.20 (6H, 2s, 2×2CH_3), 1.08 (3H, t, J= 7 Hz, CH_2CH_3), 2.32 (3H, s, NCH_3), 3.36 (2H, q, J=7 Hz, CH_2CH_3), 3.4~4.0 (5H, m, 2×CH_2 and 6α-NH), 4.00 (1H, s, 3-H), 5.17 (1H, s, 5-H), 5.59 (1H, d, J=7 Hz, 2′-H), 7.2~7.6 (5H, m, Ph), 9.40 (1H, s, 6β-NH), 9.82 (1H, d, J=7 Hz, 2′-NH). FAB-MS m/z 569 (M+H, $C_{24}H_{30}N_6O_7SNa$).

14j: HX=$(CH_3)_2NH$, yield 92%. **17j**: Yield 82%. IR ν_{max} cm^{-1} 3420, 1760, 1715, 1675, 1608, 1510, 1370, 1190. ^{1}H NMR (90 MHz, DMSO-d_6) δ 0.87 and 1.22 (6H, 2s, 2×2CH_3), 1.08 (3H, t, J= 7 Hz, CH_2CH_3), 2.28 (6H, s, $N(CH_3)_2$), 3.38 (2H, q, J=7 Hz, CH_2CH_3), 3.4~4.0 (4H, m, 2×CH_2), 3.69 (1H, s, 3-H), 5.30 (1H, s, 5-H), 5.60 (1H, d, J=8 Hz, 2′-H), 7.2~7.6 (5H, m, Ph), 9.03 (1H, s, 6β-NH), 9.75 (1H, d, J=8 Hz, 2′-NH). FAB-MS m/z 583 (M+H, $C_{25}H_{32}N_6O_7SNa$).

14k: HX=$CH_3NHOH\cdot HCl$ with NEt_3 (1 mmol), yield 61%. **17k**: Yield 79%. IR ν_{max} cm^{-1} 3400, 3300, 1765, 1714, 1676, 1609, 1511, 1395, 1187. ^{1}H NMR (250 MHz, DMSO-d_6) δ 0.83 and 1.20 (6H, 2s, 2×2CH_3), 1.08 (3H, t, J=7 Hz, CH_2CH_3), 2.56 (3H, s, NCH_3), 3.38 (2H, q, J=7 Hz, CH_2CH_3), 3.6~3.7 and 3.75~3.95 (4H, m, 2×CH_2), 3.69 (1H, s, 3-H), 5.43 (1H, s, 5-H), 5.62 (1H, d, J=8 Hz, 2′-H), 7.2~7.5 (5H, m, Ph), 8.57 (1H, br s, OH), 9.28 (1H, s, 6β-NH), 9.81 (1H, d, J=8 Hz, 2′-NH). FAB-MS m/z 585 (M+H, $C_{24}H_{30}N_6O_8SNa$).

14l: HX=$CH_3ONH_2\cdot HCl$ with NEt_3 (1 mmol), yield 61%. **17l**: Yield 90%, IR ν_{max} cm^{-1} 3400, 3300, 1767, 1715, 1677, 1611, 1507, 1397, 1187. ^{1}H NMR (250 MHz, D_2O) δ 0.91 and 1.27 (6H, 2s, 2×2CH_3), 1.18 (3H, t, J=7 Hz, CH_2CH_3), 3.50 (2H, q, J=7 Hz, CH_2CH_3), 3.60 (1H, s, OCH_3), 3.6~ 4.1 (4H, m, 2×CH_2), 4.14 (1H, s, 3-H), 5.46 and 5.47 (2H, 2s, 5-H and 2′-H), 7.3~7.6 (5H, m, Ph). FAB-MS m/z 585 (M+H, $C_{24}H_{30}N_6O_8SNa$).

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References

1) NAGARAJAN, R.; L. D. BOECK, M. GORMAN, R. L. HAMILL, C. E. HIGGENS, M. M. HOEHAN, W. M. STARK &

J. G. WHITNEY: β-Lactam antibiotics from *Streptomyces*. J. Am. Chem. Soc. 93: 2308~2310, 1971
2) STAPLEY, E. O.; M. JACKSON, S. HERNANDEZ, S. B. ZIMMERMAN, S. A. CURRIE, S. MOCHALES, J. M. MATA, H. B. WOODRUFF & D. HENDLIN: Cephamycins, a new family of β-lactam antibiotics. Antimicrob. Agents Chemother. 2: 122~131, 1972
3) HOOVER, R. R. E.: β-Lactam antibiotics: Structure-activity relationships. *In* Antibiotics Containing the β-Lactam Structure. II. *Ed.*, A. L. DEMAIN & N. A. SOLOMON, pp. 119~245, Springer-Verlag, Berlin, 1983
4) HOOVER, J. R. E.: β-Lactam antibioltics. *In* Burgers Medicinal Chemistry. 4th Ed. Part 11. *Ed.*, M. WOLFF. pp. 83~172, Wiley Interscience, New York, 1979
5) GORDON, E. M. & R. B. SYKES: Cephamycin antibiotics. *In* Chemistry and Biology of β-Lactam Antibiotics. Vol. 1. *Ed.* R. B. MORIN & M. GORMAN, pp. 199~370, Academic Press, New York, 1982
6) BENTLEY, P. H. & J. P. CLAYTON: Transformations using benzyl 6-isocyanopenicillanate. J. Chem. Soc. Perkin I 1979: 2455~2467, 1979
7) LUND, F. J.: 6β-Amidinopenicillanic acids — Synthesis and antibacterial properties. *In* Recent Advances in the Chemistry of β-Lactam Antibiotics. *Ed.* J. ELKS, pp. 25~45, First International Symposium. Cambridge, 1977
8) FEYEN, P.; H. B. KOENIG & K. G. METZGER: Penicillins and their use. Ger. Offen. 2,732,104, Jan. 25, 1979 [Chem. Abstr. 90: 137,810s, 1979]
9) CAMA, L. D. & B. G. CHRISTENSEN: Structure-activity relationships of "non-classical" β-lactam antibiotics. *In* Annual Reports in Medicinal Chemistry. *Ed.*, F. H. CLARKE, Vol. 13. pp. 149~158, 1978
10) JEN, T.; J. FRANZEE & R. R. HOOVER: A stereospecific synthesis of C-6(7) methoxypenicillin and -cephalosporin derivatives. J. Org. Chem. 38: 2857~2859, 1973
11) DALES, J. R. M. & M. A. VALANCE: Penam derivatives. Eur. Pat. Appl. EP 70,103, Jan. 19, 1983 [Chem. Abstr. 99: 87913r, 1983]
12) BIRD, A. E.; B. R. STEELE, M. O. BOLES & P. A. C. GANE: Nuclear magnetic resonance and circular dichroism of penicillins derived from dissubstituted acetic acids. J. Chem. Soc. Perkin I 1982: 563~569, 1982
13) SLOCOMBE, B.; M. J. BASKER, P. H. BENTLEY, J. P. CLAYTON, M. COLE, K. R. COMBER, R. A. DIXON, R. A. EDMONDSON, D. JACKSON, D. J. MERRIKIN & R. SUTHERLAND: BRL 17421, a novel β-lactam antibiotic, highly resistant to β-lactamases, giving high and prolonged serum levels in humans. Antimicrob. Agents Chemother. 20: 38~46, 1981
14) GOWER, J. L.; G. D. RISBRIDGER & M. J. REDRUP: Positive and negative ion-fast atom bombardment mass spectra of some penicilloic acids. J. Antibiotics 37: 33~43, 1984